COMPOSITIONS CONTAINING A COMBINATION OF A CREATINE COMPOUND AND A SECOND AGENT

Related Applications

This application is a continuation-in-part of U.S. Patent Application Serial No. 09/285,395, entitled "Compositions Containing a Combination of a Creatine Compound and a Second Agent," filed on April 2, 1999; which is a continuation-in-part of U.S. Patent Application Serial No. 09/283,267, entitled "Compositions Containing a Combination of a Creatine Compound and a Second Agent," filed on April 1, 1999; and claims priority to U.S. Provisional Application Serial No. 60/080,459, entitled "Compositions Containing a Combination of a Creatine Compound and a Second Agent," filed on April 2, 1998; the entire contents of each of the aforementioned applications are hereby incorporated herein by reference. The application is related to U.S. Provisional Application Serial No. 60/XXX,XXX, entitled "Compositions Containing A Combination of a Creatine Compound and a Second Agent," filed on October 13, 2000, the entire contents of which are hereby incorporated herein by reference. The entire contents of each of PCT/US95/14567, filed November 7, 1995, U.S. Serial No. 08/336,388, filed November 8, 1994 and U.S. Serial No. 08/853,174, filed May 7, 1997 are also hereby incorporated herein by reference.

Background of the Invention

Creatine is a compound which is naturally occurring and is found in mammalian brain and other excitable tissues, such as skeletal muscle, retina and heart. Its phosphorylated form, creatine phosphate, also is found in the same organs and is the product of the creatine kinase reaction utilizing creatine as a substrate. Creatine and creatine phosphate can be synthesized relatively easily and are believed to be non-toxic to mammals. Kaddurah-Daouk et al. (WO 92/08456 published May 29, 1992 and WO 90/09192, published August 23, 1990; U.S. 5,321,030; and U.S. 5,324,731) describe methods of inhibiting the growth, transformation and/or metastasis of mammalian cells using related compounds. Examples of compounds described by Kaddurah-Daouk et al. include cyclocreatine, b-guandidino propionic acid, homocyclocreatine, 1-carboxymethyl-2-iminohexahydropyrimidine, guanidino acetate and carbocreatine. These same inventors have also demonstrated the efficacy of such compounds for combating viral infections (U.S. 5,321,030). Elgebaly in U.S. Patent 5,091,404 discloses the use of cyclocreatine for restoring functionality in muscle tissue. Cohn in PCT publication No. WO94/16687 described a method for inhibiting the growth of several tumors using creatine and related compounds.

Neuroprotective agents can be found in nature and help to maintain an organisms ability to function without general distress to the nervous system. Often times, reduced levels below what is considered "normal" for these agents, can lead to diminished function of the nervous system.

The nervous system is an unresting assembly of cells that continually receives information, analyzes and perceives it and makes decisions. The principle cells of the nervous system are neurons and neuroglial cells. Neurons are the basic communicating units of the nervous system and possess dendrites, axons and synapses required for this role. Neuroglial cells consist of astrocytes, oligodendrocytes, ependymal cells, and microglial cells. Collectively, they are involved in the shelter and maintenance of neurons. The functions of astrocytes are incompletely understood but probably include the provision of biochemical and physical support and aid in insulation of the receptive surfaces of neurons. In addition to their activities in normal brain, they also react to CNS injury by glial scar formation. The principle function of the oligodendrocytes is the production and maintenance of CNS myelin. They contribute segments of myelin sheath to multiple axons.

The ependyma cells react to injury mainly by cell loss. Microglial cells become activated and assume the shape of a macrophage in response to injury or destruction of the brain. These cells can also proliferate and adopt a rod-like form which could surround a tiny focus of necrosis or a dead neuron forming a glial nodule. Microglial degradation of dead neurons is called neuronophagia.

The creatine kinase/creatine phosphate energy system is only one component of an elaborate energy-generating system found in nervous system cells such as, for example, neurons, oligodendrocytes and astrocytes. The components of the creatine energy system include the enzyme creatine kinase, the substrates creatine and creatine phosphate, and the transporter of creatine. The reaction catalyzed by creatine kinase is: $MgADP \pm PCr^{=} + H^{+}\ MgATP^{=} + Cr$. Some of the functions associated with this system include efficient regeneration of energy in cells with fluctuating and high energy demands, energy transport to different parts of the cell, phosphoryl transfer activity, ion transport regulation, and involvement in signal transduction pathways.

The creatine kinase/phosphocreatine system has been shown to be active in neurons, astrocytes, oligodendrocytes and Schwann cells. Manos et al., *J. Neurochem.* 56:2101-2107 (1991); Molloy et al., *J. Neurochem.* 59:1925-1932. The activity of the enzyme has been shown to be up-regulated during regeneration and down-regulated in degenerative states (see, e.g., *Annals Neurology* 35(3):331-340 (1994); DeLeon et al., *J. Neuruosci. Res.* 29:437-448 (1991); Orlovskaia et al. *Vestnik Rossiiskoi Akademii Meditsinskikh Nauk.* 8:34-39 (1992). Burbaeva et al., *Shurnal Neuropathologll Psikhiatrii Imeni S-S-Korsakova* 90(7):85-87 (1990); Mitochondrial creatine kinase was recently found to be the major constituent of pathological inclusions seen in mitochondrial myopathies. Stadhouders et al., *PNAS* 91:5080-5093 (1994).

It is an object of the present invention to provide methods for treatment of diseases that affect cells of the nervous system that utilize the creatine kinase/phosphocreatine system using compounds which modulate the system.

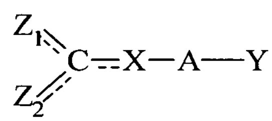
Summary of the Invention

The present invention is based, at least in part, on the discovery that certain combinations of creatine compounds and neuroprotective agents, described *infra*, can be used to treat a nervous system disease. Examples of such disease include those which there is undesired neuronal activity, characterized by undesirable demyelinating, dysmyelinating or degenerative neuronal activity in a mammal. Compositions and methods of the invention include combinations of creatine compounds and neuroprotective agents. Preferred creatine compounds include creatine, creatine phosphate, cyclocreatine, cyclocreatine phosphate, beta guanidino propionic acid, and combinations thereof. Preferred neuroprotective agents include: approved drugs for the treatment or prevention of neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet, Sinmet CR, Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate excitotoxicity inhibitors (such as glutamate uptake and biosynthesis modulation with compounds like gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors; Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants (such as pyruvate and lutein), energy enhancers (such as ribose and vincopocetine), vitamins and cofactors (such as spin traps, CoQ_{10}, carnitine, nicotinamide, Vitamin E or D) lipoic acid, vinpocetine, other fatty acids (such as docosahexanoic acid (DHA), eicosopentenoic acid (EPA), and gamma linolenic acid (GLA)), various herbal extracts (such as rosemary and

black caraway), and berry oils and meals (such as elderberry, bilberry, blackberry, blueberry, red and black raspberry).

The present invention provides methods for modulating a nervous system disease in a subject by administering to the subject a therapeutically effective amount of a combination of creatine, a creatine phosphate or a creatine analog and a neuroprotective agent, such that a nervous system disease is modulated. Additionally, or in place of the neuroprotective agent, a creatine compound can be combined with existing therapeutic drugs for neurodegenerative diseases.

The present invention also provides methods for modulating a nervous system disease in a subject by administering to the subject a therapeutically effective amount of a combination of a creatine compound and a neuroprotective agent such that a nervous system disease is modulated. The creatine compound has the formula:



and pharmaceutically acceptable salts thereof, wherein:

a) Y is selected from the group consisting of: $-CO_2H$, -NHOH, $-N0_2$, $-SO_3H$, $-C(=0)NHS0_2J$ and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C_1-C_6 straight chain alkyl, C_3-C_6 branched alkyl, C_2-C_6 alkenyl, C_3-C_6 branched alkenyl, and aryl;

b) A is selected from the group consisting of: C, CH, C_1-C_5alkyl, C_2-C_5alkenyl, C_2-C_5alkynyl, and C_1-C_5 alkoyl chain, each having 0-2 substituents which are selected independently from the group consisting of:

1) K, where K is selected from the group consisting of: C_1 -C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

2) an aryl group selected from the group consisting of: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy; and

3) -NH-M, wherein M is selected from the group consisting of: hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_1-C_4 alkoyl, C_3-C_4 branched alkyl, C_3-C_4 branched alkenyl, and C_4 branched alkoyl;

c) X is selected from the group consisting of NR_1, CHR_1, CR_1, O and S, wherein R_1 is selected from the group consisting of:

1) hydrogen;

2) K where K is selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C_5-C_9 a-amino-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;

5) a C_5-C_9 a-amino-w-aza-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon; and

6) a C_5-C_9 a-amino-w-thia-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;

d) Z_1 and Z_2 are chosen independently from the group consisting of: =0, $-NHR_2$, $-CH_2R_2$, $-NR_2OH$; wherein Z_1 and Z_2 may not both be =0 and wherein R_2 is selected from the group consisting of:

1) hydrogen;

2) K, where K is selected from the group consisting of: C_1-C_6 straight alkyl; C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C_4-C_8 a-amino-carboxylic acid attached via the w-carbon;

5) B, wherein B is selected from the group consisting of: $-CO_2H$, -NHOH, $-SO_3H$, $-N0_2$, OP(=O)(OH)(OJ) and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C_1-C_6 straight alkyl, C_3-C_6 branched alkyl, C_2-C_6 alkenyl, C_3-C_6 branched alkenyl, and aryl, wherein B is optionally connected to the nitrogen via a linker selected from the group consisting of: C_1-C_2 alkyl, C_2 alkenyl, and C_1-C_2 alkoyl;

6) -D-E, wherein D is selected from the group consisting of: C_1-C_3 straight alkyl, C_3 branched alkyl, C_2-C_3 straight alkenyl, C_3 branched alkenyl, C_1-C_3 straight alkoyl, aryl and aroyl; and E is selected from the group consisting of: $-(P0_3)_n$NMP, where n is 0-2 and NMP is ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; $-[P(=O)(OCH_3)(0)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; $-[P(=O)(OH)(CH_2)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chosen independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and $-CO_2G$, where G is independently selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1 -C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, C_4-C_6 branched alkoyl, wherein E may be attached to any point to D, and if D is alkyl or alkenyl, D may be connected at either or both ends by an amide linkage; and

7) -E, wherein E is selected from the group consisting of -$(PO_3)_n$NMP, where n is 0-2 and NMP is a ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -$[P(=O)(OCH_3)(O)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -$[P(=O)(OH)(CH_2)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chose independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO=G, where G is independently selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, C_4-C_6 branched alkoyl; and if E is aryl, E may be connected by an amide linkage;

e) if R_1 and at least one R_2 group are present, R_1 may be connected by a single or double bond to an R_2 group to form a cycle of 5 to 7 members;

f) if two R_2 groups are present, they may be connected by a single or a double bond to form a cycle of 4 to 7 members; and

g) if R_1 is present and Z_1 or Z_2 is selected from the group consisting of -NHR_2, -CH_2R_2 and -NR_2OH, then R_1 may be connected by a single or double bond to the carbon or nitrogen of either Z_1 or Z_2 to form a cycle of 4 to 7 members.

The creatine compound could be combined with a neuroprotective agent selected from the approved drugs used for the prevention or treatment of neurodegenerative diseases).

Neuroprotective agents include: approved drugs for the treatment or prevention of neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet, Sinmet CR, Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate excitotoxicity inhibitors (such as glutamate uptake and biosynthesis modulation with compounds like gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors; Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants (such as pyruvate and lutein), energy enhancers (such as ribose and vincopocetine), vitamins and cofactors (such as spin traps, CoQ_{10}, carnitine, nicotinamide, Vitamin E or D) lipoic acid, vinpocetine, other fatty acids (such as docosahexanoic acid (DHA), eicosopentenoic acid (EPA), and gamma linolenic acid (GLA)), various herbal extracts (such as rosemary and

black caraway), and berry oils and meals (such as bilberry, elderberry, english hawthorn berry, blackberry, blueberry, red and black raspberries).

The present invention further provides pharmaceutical compositions for modulating a nervous system disease in a subject. The pharmaceutical compositions include a synergistically effective amount of a combination of a creatine compound having the formula described above, a neuroprotective agent and a pharmaceutically acceptable carrier. In preferred embodiments, the creatine compound is creatine, creatine phosphate, cyclocreatine or cyclocreatine phosphate, beta guanidino propionic acid, and combinations thereof.

The present invention provides packaged nervous system disease modulators which include a creatine compound having the formula described above and at least one neuroprotective agent. Additionally, or in place of the neuroprotective agent, a creatine compound can be combined with existing therapeutic drugs for neurodegenerative diseases.

Some of the diseases susceptible to treatment with creatine compounds according to the present invention include, but are not limited to Alzheimer disease, Parkinson's disease, Huntington's disease, motor neuron disease, diabetic and toxic neuropathies, traumatic nerve injury, multiple sclerosis, acute disseminated encephalomyelitis, acute necrotizing hemorrhagic leukoencephalitis, diseases of dysmyelination, mitochondrial diseases, fungal and bacterial infections, migrainous disorders, stroke, aging, dementia, and mental disorders such as depression and schizophrenia.

The present invention also provides compositions of creatine compounds, including the formula described above, and neuroprotective agents. Preferred creatine compounds include creatine, creatine phosphate, cyclocreatine or cyclocreatine phosphate, beta guanidino propionic acid, and combinations thereof. Preferred neuroprotective agents include : approved drugs for the treatment or prevention of neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet, Sinmet CR, Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate excitotoxicity inhibitors (such as glutamate uptake and biosynthesis modulation with compounds like gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors; Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants (such as pyruvate and lutein), energy enhancers (such as ribose and vincopocetine), vitamins and cofactors

(such as spin traps, CoQ_{10}, carnitine, nicotinamide, Vitamin E or D) lipoic acid, vinpocetine, other fatty acids (such as docosahexanoic acid (DHA), eicosopentenoic acid (EPA), and gamma linolenic acid (GLA)), various herbal extracts (such as rosemary and black caraway), and berry oils and meals (such as bilberry, elderberry, english hawthorn berry, blackberry, blueberry, red and black raspberries).

The present invention further provides compositions of creatine compounds, including the formula described above, and neuroprotective agents developed as a nutritional supplement, medical food or drug form. Preferred creatine compounds include creatine, creatine phosphate, cyclocreatine, cyclocreatine phosphate, beta guanidino propionic acid, and combinations thereof. Preferred neuroprotective agents include: approved drugs for the treatment or prevention of neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet, Sinmet CR, Permax, Parlodel, Eleptyl, Symmetrel, Artane); glutamate excitotoxicity inhibitors (such as glutamate uptake and biosynthesis modulation with compounds like gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors; Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants (such as pyruvate and lutein), energy enhancers (such as ribose and vincopocetine), vitamins and cofactors (such as spin traps, CoQ_{10}, carnitine, nicotinamide, Vitamin E or D) lipoic acid, vinpocetine, other fatty acids (such as docosahexanoic acid (DHA), eicosopentenoic acid (EPA), and gamma linolenic acid (GLA)), various herbal extracts (such as rosemary and black caraway), and berry oils and meals (such as bilberry, elderberry, english hawthorn berry, blackberry, blueberry, red and black raspberries).

Brief Description of the Figures

Figure 1 is a graph illustrating the effect of creatine and cyclocreatine on lesion volumes in mice using the malonate model.

Figure 2 is a graph illustrating the dose-response effects of creatine and cyclocreatine on lesion volumes in mice using the malonate model.

Figure 3 is a graph illustrating the effect of creatine on lesion volumes in mice using the 3-NP model.

Figure 4 is a graph illustrating the effect of creatine and cyclocreatine on levels of dopamine, HVA, and DOPAC in mice using the MPTP model.

Figure 5 is a graph illustrating the dose-response effects of creatine and cyclocreatine on levels of dopamine, HVA and DOPAC in mice using the MPTP model.

Figure 6 is a graph illustrating the effect of creatine in slowing the rate of motoneural degeneration of FALS mice.

Figure 7 is a graph illustrating the effect of creatine on improving the survival times of FALS mice.

Detailed Description

The features and other details of the invention will now be more particularly described and pointed out in the claims. It will be understood that the particular embodiments of the invention are shown by way of illustration and not as limitations of the invention. The principle features of this invention can be employed in various embodiments without departing from the scope of the invention.

The methods of the present invention generally comprise administering to an individual afflicted with a disease of the nervous system a therapeutically effective amount of a creatine compound or compounds in combination with a neuroprotective agent or agents which modulate one or more of the structural or functional components of the creatine kinase/phosphocreatine system sufficient to prevent, reduce or ameliorate symptoms of the disease. Components of the system which can be modulated include the enzyme creatine kinase, the substrates creatine and creatine phosphate, and the transporter of creatine. As used herein, the term "modulate" means to change, affect or interfere with the functions of the creatine kinase system.

The present invention is based, at least in part, on the discovery that certain combinations of creatine compounds and neuroprotective agents, described *infra*, can be used to treat a nervous system disease. Examples of such diseases include those which there is undesired neuronal activity, characterized by undesirable demyelinating, dysmyelinating or degenerative neuronal activity in a mammal. Compositions and methods of the invention include combinations of creatine compounds and neuronal modulatory agents. Preferred creatine compounds include creatine, creatine phosphate, cyclocreatine, cyclocreatine phosphate, beta guanidino propionic acid and combinations

thereof. Preferred neuroprotective agents include: approved drugs for the treatment or prevention of neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet, Sinmet CR, Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate excitotoxicity inhibitors (such as glutamate uptake and biosynthesis modulation with compounds like gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors; Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants (such as pyruvate and lutein), energy enhancers (such as ribose and vincopocetine), vitamins and cofactors (such as spin traps, CoQ_{10}, carnitine, nicotinamide, Vitamin E or D) lipoic acid, vinpocetine, other fatty acids (such as docosahexanoic acid (DHA), eicosopentenoic acid (EPA), and gamma linolenic acid (GLA)), various herbal extracts (such as rosemary and black caraway), and berry oils and meals (such as elderberries, bilberries, english hawthorn berry, blackberry, blueberry, red and black raspberries). The creatine compounds could be combined with different neuroprotective agents and administered together or sequentially.

The present invention pertains to methods for modulating a nervous system disease in a subject by administering to the subject a therapeutically effective amount of a combination of creatine, a creatine phosphate or a creatine analog and a neuroprotective agent, such that a nervous system disease is modulated. Additionally, or in place of the neuroprotective agent, a creatine compound can be combined with existing therapeutic drugs for neurodegenerative diseases.

Creatine compounds which are particularly effective for this purpose include creatine, creatine phosphate, and analogs thereof which are described in detail below. The term "creatine compounds" will be used herein to include creatine, creatine phosphate, and compounds which are structurally similar to creatine or creatine phosphate, analogs of creatine and creatine phosphate, and combinations thereof. The term "creatine compounds" also includes compounds which "mimic" the activity of creatine, creatine phosphate or creatine analogs, i.e., compounds which inhibit or modulate the creatine kinase system. The term creatine compound is also intended to include pharmaceutically acceptable or physiologically acceptable salts of the compounds. Creatine compounds have previously been described in copending application Ser. No. 07/061,677 entitled Methods of Treating Body Parts Susceptible to Ischemia Using Creatine Analogs, filed May 14, 1993; copending application Ser. No. 08/009,638 entitled Creatine Phosphate, Creatine Phosphate Analogs and Uses Therefor, filed on Jan. 27, 1993; copending application Ser. No. 07/812,561 entitled

Creatine Analogs Having Antiviral Activity, filed Dec. 20, 1991; and copending application Ser. No. 07/610,418 entitled Method of Inhibiting transformation of Cells in Which Purine Metabolic Enzyme Activity is Elevated, filed Nov. 7, 1990. The entire contents of each of the copending applications are herein expressly incorporated by reference, along with their published foreign counterparts; and all of the creatine compounds along with their methods of synthesis and discussed in the aforementioned applications are intended to be part of this invention unless specifically stated otherwise.

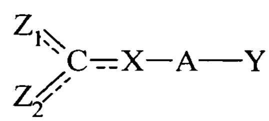
The term "mimics" is intended to include compounds which may not be structurally similar to creatine but mimic the therapeutic activity of creatine, creatine phosphate or structurally similar compounds. The term "inhibitors of creatine kinase system" are compounds which inhibit the activity of the creatine kinase enzyme, molecules that inhibit the creatine transporter or molecules that inhibit the binding of the enzyme to other structural proteins, enzymes or lipids. The term "modulators of the creatine kinase system" are compounds which modulate the activity of the enzyme, or the activity of the transporter of creatine or the ability of other proteins or enzymes or lipids to interact with the system. The term "creatine analog" is intended to include compounds which are structurally similar to creatine or creatine phosphate, compounds which are art-recognized as being analogs of creatine or creatine phosphate, and/or compounds which share the same or similar function as creatine or creatine phosphate.

The language "modulating a nervous system disease" or "modulating a disease of the nervous system" is intended to include prevention of the disease, amelioration and/or arrest of a preexisting disease, or the elimination of a preexisting disease. The combinations of creatine analogs and neuroprotective agents described herein have both curative and prophylactic effects on disease development and progression.

The language "therapeutically effective amount" is intended to include the amount of a combination of a creatine compound and neuroprotective agent sufficient to prevent onset of diseases of the nervous system or significantly reduce progression of such diseases in the subject being treated. A therapeutically effective amount can be determined on an individual basis and will be based, at least in part, on consideration of the severity of the symptoms to be treated and the activity of the specific analog selected if an analog is being used. Further, the effective amounts of

the creatine compound(s) and neuroprotective agent(s) may vary according to the age, sex and weight of the subject being treated. Thus, a therapeutically effective amount of the combinations can be determined by one of ordinary skill in the art employing such factors as described above using no more than routine experimentation in clinical management.

The present invention also pertains to methods for modulating a nervous system disease in a subject by administering to the subject a therapeutically effective amount of a combination of a creatine compound and a neuroprotective agent such that a nervous system disease is modulated. The creatine compound has the formula:



and pharmaceutically acceptable salts thereof, wherein:

a) Y is selected from the group consisting of: $-CO_2H$, -NHOH, $-N0_2$, $-SO_3H$, $-C(=0)NHS0_2J$ and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C_1-C_6 straight chain alkyl, C_3-C_6 branched alkyl, C_2-C_6 alkenyl, C_3-C_6 branched alkenyl, and aryl;

b) A is selected from the group consisting of: C, CH, C_1-C_5alkyl, C_2-C_5alkenyl, C_2-C_5alkynyl, and C_1-C_5 alkoyl chain, each having 0-2 substituents which are selected independently from the group consisting of:

1) K, where K is selected from the group consisting of: C_1 -C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

2) an aryl group selected from the group consisting of: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy; and

3) -NH-M, wherein M is selected from the group consisting of: hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_1-C_4 alkoyl, C_3-C_4 branched alkyl, C_3-C_4 branched alkenyl, and C_4 branched alkoyl;

c) X is selected from the group consisting of NR_1, CHR_1, CR_1, O and S, wherein R_1 is selected from the group consisting of:

1) hydrogen;

2) K where K is selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C_5-C_9 a-amino-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;

5) a C_5-C_9 a-amino-w-aza-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon; and

6) a C_5-C_9 a-amino-w-thia-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;

d) Z_1 and Z_2 are chosen independently from the group consisting of: =0, $-NHR_2$, $-CH_2R_2$, $-NR_2OH$; wherein Z_1 and Z_2 may not both be =0 and wherein R_2 is selected from the group consisting of:

1) hydrogen;

2) K, where K is selected from the group consisting of: C_1-C_6 straight alkyl; C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl,

C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: -CH_2L and -$COCH_2$L where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C_4-C_8 a-amino-carboxylic acid attached via the w-carbon;

5) B, wherein B is selected from the group consisting of: -CO_2H, -NHOH, -SO_3H, -$N0_2$, OP(=O)(OH)(OJ) and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C_1-C_6 straight alkyl, C_3-C_6 branched alkyl, C_2-C_6 alkenyl, C_3-C_6 branched alkenyl, and aryl, wherein B is optionally connected to the nitrogen via a linker selected from the group consisting of: C_1-C_2 alkyl, C_2 alkenyl, and C_1-C_2 alkoyl;

6) -D-E, wherein D is selected from the group consisting of: C_1-C_3 straight alkyl, C_3 branched alkyl, C_2-C_3 straight alkenyl, C_3 branched alkenyl, C_1-C_3 straight alkoyl, aryl and aroyl; and E is selected from the group consisting of: -$(P0_3)_n$NMP, where n is 0-2 and NMP is ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -$[P(=O)(OCH_3)(0)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -$[P(=O)(OH)(CH_2)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chosen independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO_2G, where G is independently selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1 -C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, C_4-C_6 branched alkoyl, wherein E may be attached to any point to D, and if D is alkyl or alkenyl, D may be connected at either or both ends by an amide linkage; and

7) -E, wherein E is selected from the group consisting of -$(P0_3)_n$NMP, where n is 0-2 and NMP is a ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -$[P(=O)(OCH_3)(0)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -$[P(=O)(OH)(CH_2)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected

via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chose independently from the group consisting of: C_l, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO=G, where G is independently selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, C_4-C_6 branched alkoyl; and if E is aryl, E may be connected by an amide linkage;

e) if R_1 and at least one R_2 group are present, R_1 may be connected by a single or double bond to an R_2 group to form a cycle of 5 to 7 members;

f) if two R_2 groups are present, they may be connected by a single or a double bond to form a cycle of 4 to 7 members; and

g) if R_1 is present and Z_1 or Z_2 is selected from the group consisting of -NHR_2, -CH_2R_2 and -NR_2OH, then R_1 may be connected by a single or double bond to the carbon or nitrogen of either Z_1 or Z_2 to form a cycle of 4 to 7 members.

Additionally, or in place of the neuroprotective agent, a creatine compound can be combined with existing therapeutic drugs for neurodegenerative diseases.

The term "neuroprotective agent" is intended to include those compositions which prevent depletion of ATP prevent glutamate excitotoxicity or prevent production of free radicals or other agents which interfere with, destroy, or diminish nervous system activity. Representative neuroprotective agents include approved drugs for the treatment or prevention of neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet, Sinmet CR, Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate excitotoxicity inhibitors (such as glutamate uptake and biosynthesis modulation with compounds like gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors; Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants (such as pyruvate and lutein), energy enhancers (such as ribose and vincopocetine), vitamins and cofactors (such as spin traps, CoQ_{10}, carnitine, nicotinamide, Vitamin E or D) lipoic acid, vinpocetine, other fatty acids (such as docosahexanoic acid (DHA), eicosopentenoic acid (EPA), and gamma linolenic acid (GLA)), various herbal extracts (such as rosemary and black caraway), and berry oils and meals (such as bilberry, elderberry, english hawthorn berry, blackberry, blueberry, red and black raspberries).

The present invention further pertains to pharmaceutical compositions for modulating a nervous system disease in a subject. The pharmaceutical compositions include an effective amount, e.g. synergistically effective amount, of a combination of a creatine compound having the formula described above, a neuroprotective agent and a pharmaceutically acceptable carrier. In preferred embodiments, the creatine compound is creatine, creatine phosphate, cyclocreatine or cyclocreatine phosphate beta guanidino propionic acid.

The present invention also pertains to packaged nervous system disease modulators which include a creatine compound having the formula described above and at least one neuroprotective agent. Additionally, or in place of the neuroprotective agent, a creatine compound can be combined with existing therapeutic drugs for neurodegenerative diseases.

The language "pharmaceutically acceptable carrier" is intended to include substances capable of being coadministered with the creatine compound(s) and neuroprotective agent(s) and which allows the active ingredients to perform their intended function of preventing, ameliorating, arresting, or eliminating a disease(s) of the nervous system. Examples of such carriers include agents to enhance creatine compound uptake such as sugars, solvents, dispersion media, adjuvants, delay agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Any conventional media and agent compatible with the creatine compound may be used within this invention.

The term "pharmaceutically acceptable salt" is intended to include art-recognized pharmaceutically acceptable salts. Typically these salts are capable of being hydrolyzed under physiological conditions. Examples of such salts include sodium, potassium and hemisulfate. The term further is intended to include lower hydrocarbon groups capable of being hydrolyzed under physiological conditions, i.e. groups which esterify the carboxyl moiety, e.g. methyl, ethyl and propyl.

The term "subject" is intended to include living organisms susceptible to having diseases of the nervous system, e.g. mammals. Examples of subjects include humans, dogs, cats, horses, cows, goats, rats and mice. The term "subject" further is intended to include transgenic species.

The present invention pertains to compositions of creatine compounds, including the formula described above, and neuroprotective agents improved nervous system function. Preferred creatine compounds include creatine, creatine phosphate, cyclocreatine or cyclocreatine phosphate beta guanidino propionic acid. Preferred neuroprotective agents include: approved drugs for the treatment or prevention of neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet, Sinmet CR, Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate excitotoxicity inhibitors (such as glutamate uptake and biosynthesis modulation with compounds like gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors; Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants (such as pyruvate and lutein), energy enhancers (such as ribose and vincopocetine), vitamins and cofactors (such as spin traps, CoQ_{10}, carnitine, nicotinamide, Vitamin E or D) lipoic acid, vinpocetine, other fatty acids (such as docosahexanoic acid (DHA), eicosopentenoic acid (EPA), and gamma linolenic acid (GLA)), various herbal extracts (such as rosemary and black caraway), and berry oils and meals (such as bilberry, elberberry, english hawthorn berry, blackberry, blueberry, red and black raspberries).

These compositions of creatine compounds and neuroprotective agents can be used as dietary food supplements or medical foods to improve nervous system activities and associated functions. When used as a dietary food supplement or a medical food, these compositions are included as additives to enhance the ability of the food to protect, alleviate, and/or enhance the nervous system against nervous system disease states.

The language "diseases of the nervous system" or "nervous system disease" is intended to include diseases of the nervous system whose onset, amelioration, arrest, or elimination is effectuated by the creatine compounds described herein. Examples of types of diseases of the nervous system include demyelinating, dysmyelinating and degenerative diseases. Examples of locations on or within the subject where the diseases may originate and/or reside include both central and peripheral loci. As the term "disease" is used herein, it is understood to exclude, and only encompass maladies distinct from, neoplastic pathologies and tumors of the nervous system, inschemic injury and viral infections of the nervous system. Examples of types of diseases suitable for treatment with the methods and compounds of the instant invention are discussed in detail below.

Diseases of the Nervous System

Diseases of the nervous system fall into two general categories: (a) pathologic processes such as infections, trauma and neoplasma found in both the nervous system and other organs; and, (b) diseases unique to the nervous system which include diseases of myelin and systemic degeneration of neurons.

Of particular concern to neurologists and other nervous system practitioners are diseases of: (a) demyelination which can develop due to infection, autoimmune antibodies, and macrophage destruction; and, (b) dysmyelination which result from structural defects in myelin.

Diseases of neurons can be the result of: (a) aberrant migration of neurons during embryogenesis and early stage formation; or (b) degenerative diseases resulting from a decrease in neuronal survival, such as occurs in, for example, Alzheimer's disease, Parkinson's disease, Huntington's disease, motor neuron disease, ischemia-related disease and stroke, and diabetic neuropathy.

Demyelinating Diseases:

Primary demyelination is a loss of myelin sheaths with relative preservation of the demyelinated axons. It results either from damage to the oligodendroglia which make the myelin or from a direct, usually immunologic or toxic attack on the myelin itself. Secondary demyelination, in contrast, occurs following axonal degeneration. The demyelinating diseases are a group of CNS conditions characterized by extensive primary demyelination. They include multiple sclerosis and its variants and perivenous encephalitis. There are several other diseases in which the principal pathologic change is primary demyelination, but which are usually conveniently classified in other categories such as inborn errors of metabolism, the leukodystrophies, viral disease (progressive multifocal leukoencephalopathy PM), as well as several other rare disorders of unclear etiology.

Multiple Sclerosis (MS)

Multiple sclerosis is a disease of the central nervous system (CNS) that has a peak onset of 30-40 years. It affects all parts of the CNS and causes disability related to visual, sensory, motor, and cerebellar systems. The disease manifestations can be mild and intermittent or progressive and devastating.

The pathogenesis is due to an autoimmune attack on CNS myelin. The treatments available are symptomatic treating spasticity, fatigue, bladder dysfunction, and spasms. Other treatments are directed towards stopping the immunologic attack on myelin. These consist of corticosteroids such as prednisone and methylprednisolone, general immunosuppressants such as cyclophosphamide and azathioprine, and immunomodulating agents such as beta-interferon. No treatments are available to preserve myelin or make it resistant to attacks.

Acute Disseminated Encephalomyelitis

Acute Disseminated Encephalomyelitis usually occurs following a viral infection and is thought to be due to an autoimmune reaction against CNS myelin, resulting in paralysis, lethargy, and coma. It differs from MS by being a monophasic disease whereas MS is characterized by recurrence and chronicity. Treatment consists of administration of steroids.

Acute Necrotizing Hemorrhagic Leukoencephalitis

This is a rare disease that is generally fatal. It is also thought to be mediated by autoimmune attack on CNS myelin that is triggered by a viral infection. Neurologic symptoms develop abruptly with headache, paralysis and coma. Death usually follows within several days. Treatment is supportive.

Leukodystrophies

These are diseases of the white matter resulting from an error in the myelin metabolism that leads to impaired myelin formation. They are thought of as dysmyelinating diseases, and can become manifest at an early age.

Metachromatic Leukodystrophy: an autosomal recessive (inherited) disorder due to deficiency of the enzyme arylsulfatase A leading to accumulation of lipids. There is demyelination in the CNS and peripheral nervous system leading to progressive weakness and spasticity.

Krabbe's disease: Also inherited as autosomal recessive and due to deficiency of another enzyme: galactocerebroside beta-galactosidase.

Adrenoleukodystrophy and adrenomyeloneuropathy: affect the adrenal glad in addition to the nervous system.

No treatment is available to any of the leukodystrophies except for supportive treatment

Degenerative Diseases:

There is no good etiology or pathophysiology known for these diseases, and no compelling reason to assume that they all have a similar etiology. Diseases under this category have general similarities. They are diseases of neurons that tend to result in selective impairment, affecting one or more functional systems of neurons while leaving others intact.

Parkinson's Disease:

Parkinson's disease is due to loss of dopaminergic neurones in the substantia nigra of the brain. It is manifested by slowed voluntary movements, rigidity, expressionless face and stooped posture. Several drugs are available to increase dopaminergic function such as levodopa, carbidopa, bromocriptine, pergolide, or decrease cholinergic function such as benztropine, and amantadine. Selegiline is a new treatment designed to protect the remaining dopaminergic neurons.

Spinocerebellar Degenerations

This is a group of degenerative diseases that affects in varying degrees the basal ganglia, brain stem, cerebellum, spinal cord, and peripheral nerves. Patients present symptoms of Parkinsonism, ataxia, spasticity, and motor and sensory deficits reflecting damage to different anatomic areas and/or neuronal systems in the CNS.

Degenerative Disease Affecting Motor Neurons

Included in this category are diseases such as amyotrophic lateral sclerosis (ALS), and spinal muscular atrophy. They are characterized by degeneration of motor neurones in the CNS leading to progressive weakness, muscle atrophy, and death caused by respiratory failure. Treatments are only symptomatic, there are no available treatments to slow down or stop the disease.

Alzheimer Disease (AD):

This disease is characterized clinically by slow erosion of mental function, culminating in profound dementia. The diagnostic pathologic hallmark of AD is the presence of large numbers of senile plagues and neurofibrillary tangles in the brain especially in neocortex and hippocampus. Loss of specific neuron populations in these brain regions and in several subcortical nuclei correlates with depletion in

certain neurotransmitters including acetylcholine. The etiology of AD is still unknown. To date a lot of research has focused on the composition and genesis of the B/A4 amyloid component of senile plagues. Alzheimer's disease is characterized clinically by the slow erosion of intellectual function with the development of profound dementia. There are no treatments that slow the progression.

Huntington Disease (HD):

HD is an autosomal dominant disorder of midlife onset, characterized clinically by movement disorder, personality changes, and dementia often leading to death in 15-20 years. The neuropathologic changes in the brain are centered in the basal ganglia. Loss of a class of projection neurons, called "spiny cells" because of their prominent dendritic spinous processes, is typical. This class of cells contains gamma-aminobutyric acid (GABA), substance P, and opioid peptides. Linkage studies have localized the gene for HD to the most distal band of the short arm of chromosome 4. No treatments are available that have been shown to retard progression of the disease. Experimental studies showing a similarity between neurons that are susceptible to N-methyl d-aspartate (NMDA) agonists and those that disappear in HD has led to encouraging speculation that NMDA antagonists might prove beneficial. Some recent studies suggest that a defect in brain energy metabolism might occur in HD and enhance neuronal vulnerability to excitotoxic stress.

Mitochondrial Encephalomyopathies:

Mitochondrial encephalomyopathies are a heterogenous group of disorders affecting mitochondrial metabolism. These deficits could involve substrate transport, substrate utilization, defects of the Krebs Cycle, defects of the respiratory chain, and defects of oxidation/phosphorylation coupling. Pure myopathies vary considerably with respect to age at onset, course (rapidly progressive, static, or even reversible), and distribution of weakness (generalized with respiratory failure, proximal more than distal facioscapulohumeral, orbicularis and extraocular muscles with ptosis and progressive external ophthalmoplegia). Patients with mitochondrial myopathies complain of exercise intolerance and premature fatigue.

Peripheral Nervous System Disorders

The peripheral nervous system (PNS) consists of the motor and sensory components of the cranial and spinal nerves, the autonomic nervous system with its sympathetic and parasympathetic divisions, and the peripheral ganglia. It is the

conduit for sensory information to the CNS and effector signals to the peripheral organs such as muscle. Contrary to the brain, which has no ability to regenerate, the pathologic reactions of the PNS include both degeneration and regeneration. There are three basic pathological processes: Wallerian degeneration, axonal degeneration and segmental demyelination that could take place.

Some of the neuropathic syndromes include:

Acute ascending motor paralysis with variable sensory disturbance; examples being acute demyelinating neuropathics, infectious mononucleosis with polyneuritis, hepatitis and polyneuritis, toxic polyneuropathies.

Subacute sensorimotor polyneuropathy; examples of acquired axonal neurophathics include paraproteinemias, uremia diabetes, amyloidosis, connective tissue diseases and leprosy. Examples of inherited diseases include mostly chronic demyelination with hypertrophic changes, such as peroneal muscular atrophy, hypertrophic polyneuropathy and Refsum's diseases.

Chronic relapsing polyneuropathy; such as idiopathic polyneuritis porphyria, Beriberi and intoxications.

Mono or multiple neuropathy; such as pressure palsies, traumatic palsies, serum neuritis, zoster and leprosy.

Aging:

During the process of aging increased oxidative damage and impaired mitochondrial functions contribute to neuronal cell death. Mitochondria are deeply involved in the production of reactive oxygen species and are themselves highly susceptible to oxidative stress which results in apoptotic cell death. Accumulation of mutations in the mitochondrial DNA seems to contribute to the process of aging as evident by respiratory chain function defects and mutations in mDNA with aging.

The methods and compounds of this invention can also be used to treat neuromuscular disorders and epilepsy.

Creatine Compounds Useful For TreatingNervous System Diseases

Creatine compounds useful in the present invention include compounds which modulate one or more of the structural or functional components of the creatine kinase/phosphocreatine system. Compounds which are effective for this purpose include creatine, creatine phosphate and analogs thereof, compounds which mimic their activity, and salts of these compounds as defined above. Exemplary creatine compounds are described below.

Creatine (also known as N-(aminoiminomethyl)-N-methylglycine; methylglycosamine or N-methyl-guanido acetic acid) is a well-known substance. (See, The Merck Index, Eleventh Edition, No. 2570 (1989).

Creatine is phosphorylated chemically or enzymatically by creatine kinase to generate creatine phosphate, which also is well-known (see, The Merck Index, No. 7315). Both creatine and creatine phosphate (phosphocreatine) can be extracted from animal tissue or synthesized chemically. Both are commercially available.

Cyclocreatine is an essentially planar cyclic analog of creatine. Although cyclocreatine is structurally similar to creatine, the two compounds are distinguishable both kinetically and thermodynamically. Cyclocreatine is phosphorylated efficiently by creatine kinase in the forward reaction both *in vitro* and *in vivo* (Rowley, G.L., *J. Am. Chem. Soc.* 93: 5542-5551 (1971); McLaughlin, A.C. et. al., *J. Biol. Chem.* 247:4382-4388 (1972)).

The phosphorylated compound phosphocyclocreatine is structurally similar to phosphocreatine; however, the phosphorous-nitrogen (P-N) bond of cyclocreatine phosphate is more stable than that of phosphocreatine. LoPresti, P. and M. Cohn, *Biochem. Biophys. Acta* 998:317-320 (1989); Annesley, T. M. and J. B. Walker, *J. Biol. Chem.* 253:8120-8125 (1978); Annesley, T.M. and J.B. Walker, *Biochem. Biophys. Res. Commun.* 74:185-190 (1977).

Creatine analogs and other agents which act to interfere with the activity of creatine biosynthetic enzymes or with the creatine transporter are useful in the present method of treating nervous system diseases. In the nervous system, there are many possible intracellular, as well as extracellular, sites for the action of compounds that inhibit, increase, or otherwise modify, energy generation through brain creatine

kinase and/or other enzymes which are associated with it. Thus the effects of such compounds can be direct or indirect, operating by mechanisms including, but not limited to, influencing the uptake or biosynthesis of creatine, the function of the creatine phosphate shuttle, inhibiting the enzyme activity, or the activity of associated enzymes, or altering the levels of substrates or products of a reaction to alter the velocity of the reaction.

Substances known or believed to modify energy production through the creatine kinase/phosphocreatine system which can be used in the present method are described below. Exemplary compounds are shown in Tables 1 and 2.

TABLE 1

CREATINE ANALOGS

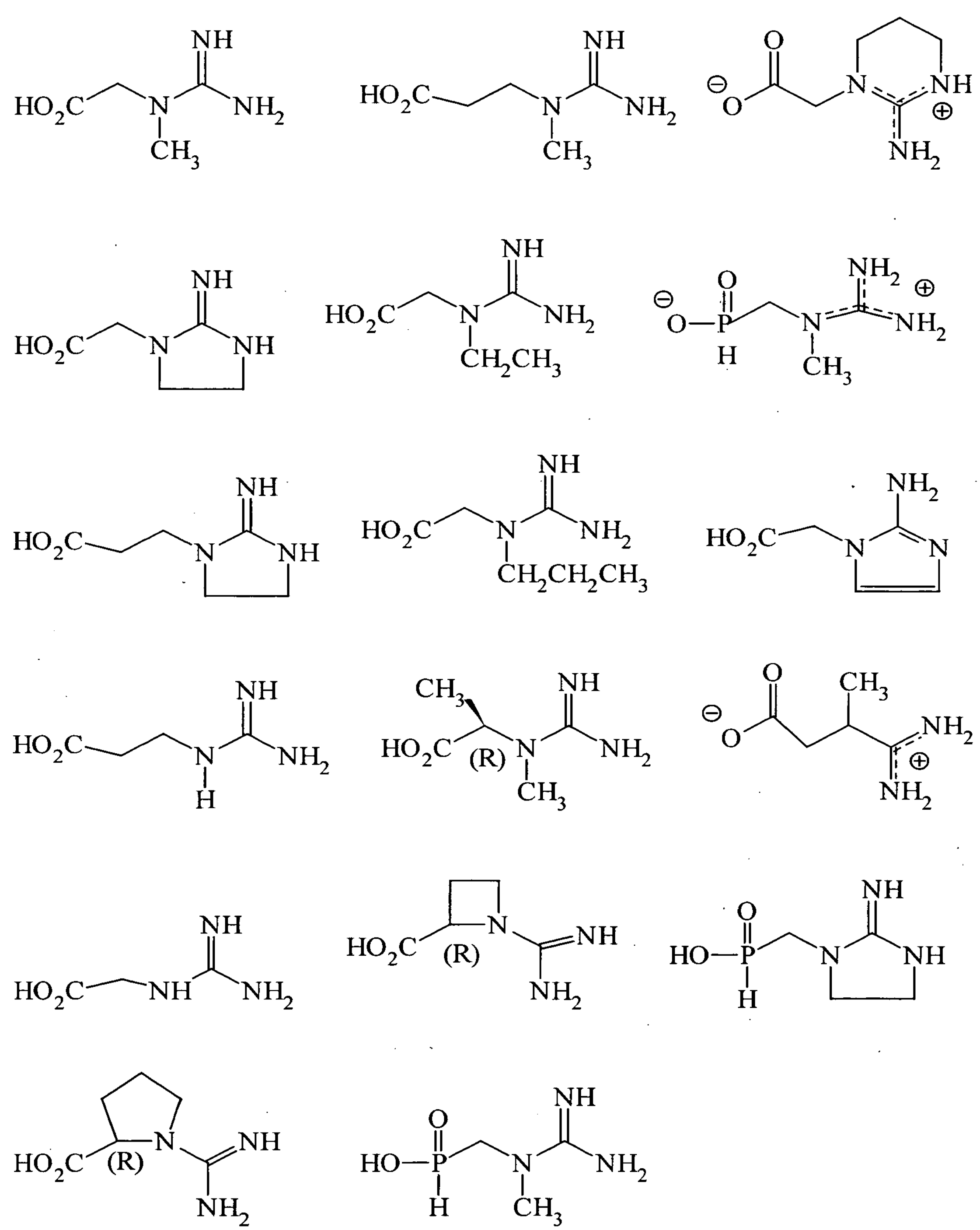
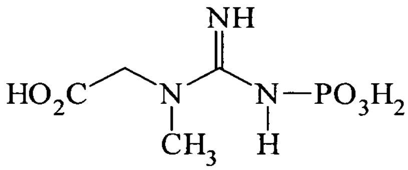
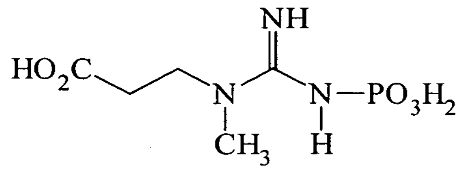
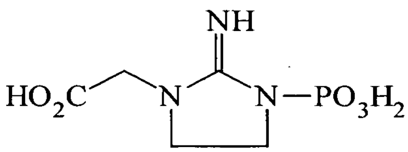


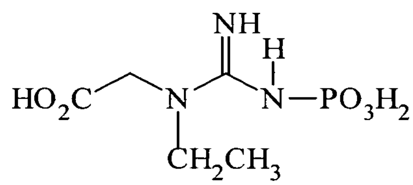
TABLE 2

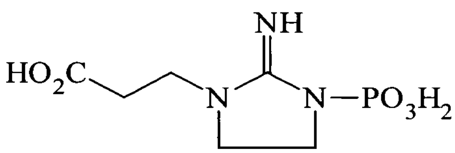
CREATINE ANALOGS

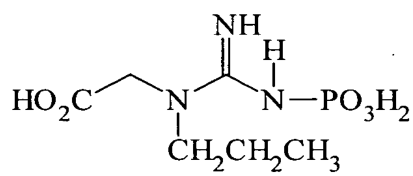


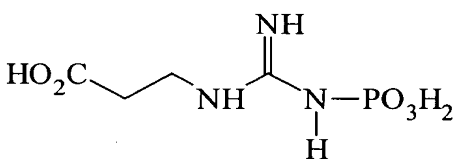


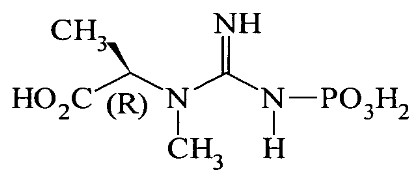


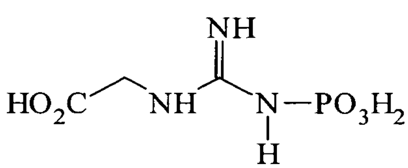


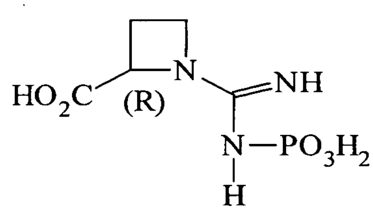


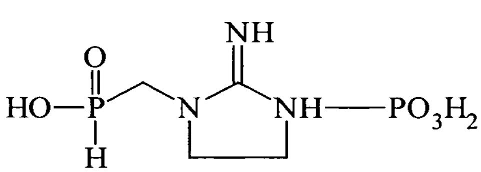


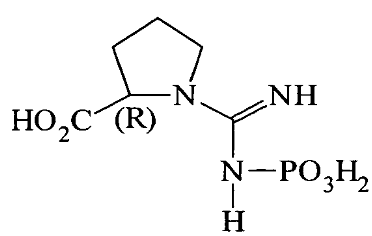


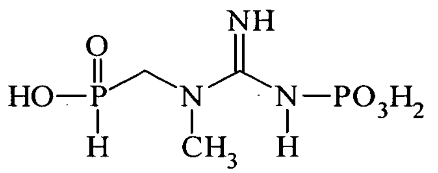


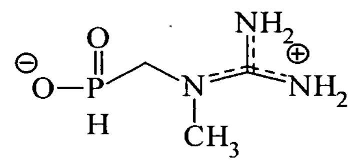


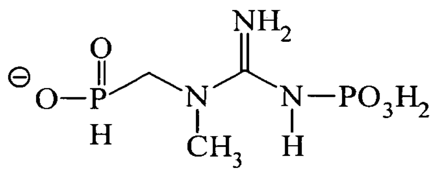












It will be possible to modify the substances described below to produce analogs which have enhanced characteristics, such as greater specificity for the enzyme, enhanced stability, enhanced uptake into cells, or better binding activity.

Compounds which modify the structure or function of the creatine kinase/creatine phosphate system directly or indirectly are useful in preventing and/or treating diseases of the nervous system characterized by up regulation or down regulation of the enzyme system.

In diseases where the creatine kinase/creatine phosphate system is down regulated, for example, uncontrolled firing of neurons, molecules useful for treating these diseases include those that will up regulate the activity, or could support energy (ATP) production for a longer period of time. Examples include creatine phosphate and related molecules that form stable phosphagens which support ATP production over a long period of time.

In diseases where the creatine kinase/creatine phosphate system is up regulated, the molecules that are useful include those that will down regulate the activity and/or inhibit energy production (ATP).

Molecules that regulate the transporter of creatine, or the association of creatine kinase with other protein or lipid molecules in the membrane, the substrates concentration creatine and creatine phosphate also are useful in preventing and/or treating diseases of the nervous system.

Compounds which are useful in the present invention can be inhibitors, substrates or substrate analogs, of creatine kinase, which when present, could modify energy generation or high energy phosphoryl transfer through the creatine kinase/phosphocreatine system. In addition, modulators of the enzymes that work in conjunction with creatine kinase now can be designed and used, individually, in combination or in addition to other drugs, to make control of the effect on brain creatine kinase tighter.

The pathways of biosynthesis and metabolism of creatine and creatine phosphate can be targeted in selecting and designing compounds which modify energy production or high energy phosphoryl transfer through the creatine kinase system. Compounds targeted to specific steps may rely on structural analogies with either creatine or its precursors. Novel creatine analogs differing from creatine by substitution, chain extension, and/or cyclization may be designed. The substrates of multisubstrate enzymes may be covalently linked, or analogs which mimic portions of the different substrates may be designed. Non-hydrolyzable phosphorylated analogs can also be designed to mimic creatine phosphate without sustaining ATP production.

A number of creatine and creatine phosphate analogs have been previously described in the literature or can be readily synthesized. Examples are these shown in Table I and Table 2. Some of them are slow substrates for creatine kinase.

Tables 1 and 2 illustrate the structures of creatine, cyclocreatine (1-carboxymethyl-2-iminoimidazolidine), N-phosphorocreatine (N-phosphoryl creatine), cyclocreatine phosphate (3-phosphoryl-1-carboxymethyl-2-iminoimidazolidine) and other compounds. In addition, 1-carboxymethyl-2-aminoimidazole, 1-carboxymethyl-2, 2-iminomethylimidazolidine, 1-carboxyethyl-2-iminoimidazolidine, N-ethyl-N-amidinoglycine and b-guanidinopropionic acid are believed to be effective.

Cyclocreatine (1-carboxymethyl-2-iminoimidazolidine) is an example of a class of substrate analogs of creatine kinase, which can be phosphorylated by creatine kinase and which are believed to be active.

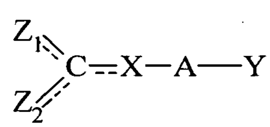
A class of creatine kinase targeted compounds are bi-substrate analogs comprising an adenosine-like moiety linked via a modifiable bridge to a creatine link

moiety (i.e., creatine or a creatine analog). Such compounds are expected to bind with greater affinity than the sum of the binding interaction of each individual substrate (e.g., creatine and ATP). The modifiable bridge linking an adenosine like moiety at the 5' carbon to a creatine like moiety can be a carbonyl group, alkyl (a branched or straight chain hydrocarbon group having one or more carbon atoms), or substituted alkyl group (an alkyl group bearing one or more functionalities, including but not limited to unsaturation, heteroatom substituents, carboxylic and inorganic acid derivatives, and electrophilic moieties).

Another class of potential compounds for treating nervous system disorders is designed to inhibit (reversibly or irreversibly) creatine kinase. The analogs of creatine in this class can bind irreversibly to the active site of the enzyme. Two such affinity reagents that have previously been shown to completely and irreversibly inactivate creatine kinase are epoxycreatine Marietta, M.A. and G.L. Kenyon *J. Biol Chem.* 254: 1879-1886 (1979)) and isoepoxycreatine. There are several approaches to enhancing the specificity and hence, the efficacy of active site-targeted irreversible inhibitors of creatine kinase, incorporating an electrophilic moiety. The effective concentration of a compound required for inhibition can be lowered by increasing favorable and decreasing unfavorable binding contacts in the creatine analog.

N-phosphorocreatine analogs also can be designed which bear non-transferable moieties which mimic the N-phosphoryl group. These cannot sustain ATP production.

Some currently preferred creatine compounds of this invention are those encompassed by the general formula I:



and pharmaceutically acceptable salts thereof, wherein:

a) Y is selected from the group consisting of: $-CO_2H$-NHOH, $-NO_2$, $-SO_3H$, $-C(=O)NHSO_2J$ and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C_1-C_6 straight chain alkyl, C_3-C_6 branched alkyl, C_2-C_6 alkenyl, C_3-C_6 branched alkenyl, and aryl;

b) A is selected from the group consisting of: C, CH, C_1-C_5alkyl, C_2-C_5alkenyl, C_2-C_5alkynyl, and C_1-C_5alkoyl chain, each having 0-2 substituents which are selected independently from the group consisting of:

1) K, where K is selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

2) an aryl group selected from the group consisting of: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy; and

3) -NH-M, wherein M is selected from the group consisting of: hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_1-C_4 alkoyl, C_3-C_4 branched alkyl, C_3-C_4 branched alkenyl, and C_4 branched alkoyl;

c) X is selected from the group consisting of NR_1, wherein R_1 is selected from the group consisting of:

1) hydrogen;

2) K where K is selected from the group consisting of: C_1 -C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a Cs-Cg a-amino-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;

5) 2 Cs-Cg a-amino-w-aza-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon; and

6) a Cs-Cg a-amino-w-thia-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;

d) Z_1 and Z_2 are chosen independently from the group consisting of: =O, $-NHR_2$, $-CH_2R_2$, $-NR_2OH$; wherein Z_1 and Z_2 may not both be =0 and wherein R_2 is selected from the group consisting of:

1) hydrogen;

2) K, where K is selected from the group consisting of: C_1-C_6 straight alkyl; C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, and C_4-C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) 2 C_4-C_8 a-amino-carboxylic acid attached via the w-carbon;

5) B, wherein B is selected from the group consisting of: $-CO_2H$-NHOH, $-SO_3H$, $-NO_2$, OP(=O)(OH)(OJ) and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C_1-C_6 straight alkyl, C_3-C_6 branched alkyl, C_2-C_6 alkenyl, C_3-C_6 branched alkenyl, and aryl, wherein B is optionally connected to the nitrogen via a linker selected from the group consisting of: C_1-C_2 alkyl, C_2 alkenyl, and C_1-C_2 alkoyl;

6) -D-E, wherein D is selected from the group consisting of: C_1-C_3 straight alkyl, C_3 branched alkyl, C_2-C_3 straight alkenyl, C_3 branched alkenyl, C_1 -C_3 straight alkoyl, aryl and aroyl; and E is selected from the group consisting of: -$(PO_3)_n$NMP, where n is 0-2 and NMP is ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -$[P(=O)(OCH_3)(O)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -$[P(=O)(OH)(CH_2)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chosen independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO_2G, where G is independently selected from the group consisting of: C_1 -C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, C_4-C_6 branched alkoyl, wherein E may be attached to any point to D, and if D is alkyl or alkenyl, D may be connected at either or both ends by an amide linkage; and

7) -E, wherein E is selected from the group consisting of -$(PO_3)_n$NMP, where n is 0-2 and NMP is a ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -$[P(=O)(OCH_3)(O)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -$[P(=O)(OH)(CH_2)]_m$-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chose independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO_2G, where G is independently selected from the group consisting of: C_1-C_6 straight alkyl, C_2-C_6 straight alkenyl, C_1-C_6 straight alkoyl, C_3-C_6 branched alkyl, C_3-C_6 branched alkenyl, C_4-C_6 branched alkoyl; and if E is aryl, E may be connected by an amide linkage;

e) if R_1 and at least one R_2 group are present, R_1 may be connected by a single or double bond to an R_2 group to form a cycle of 5 to 7 members;

f) if two R_2 groups are present, they may be connected by a single or a double bond to form a cycle of 4 to 7 members; and

g) if R_1 is present and Z_1 or Z_2 is selected from the group consisting of -NHR2, -CH_2R_2 and -NR_2OH, then R_1 may be connected by a single or double bond to the carbon or nitrogen of either Z_1 or Z_2 to form a cycle of 4 to 7 members.

Creatine, creatine phosphate and many creatine analogs, and competitive inhibitors are commercially available. Additionally, analogs of creatine may be synthesized using conventional techniques. For example, creatine can be used as the starting material for synthesizing at least some of the analogs encompassed by formula I. Appropriate synthesis reagents, e.g. alkylating, alkenylating or alkynylating agents may be used to attach the respective groups to target sites. Alternatively, reagents capable of inserting spacer groups may be used to alter the creatine structure. Sites other than the target site are protected using conventional protecting groups while the desired sites are being targeted by synthetic reagents.

If the creatine analog contains a ring structure, then the analog may be synthesized in a manner analogous to that described for cyclocreatine (Wang, T., *J. Org. Chem.* 39:3591-3594 (1974)). The various other substituent groups may be introduced before or after the ring is formed.

Many creatine analogs have been previously synthesized and described (Rowley et al., *J. Am. Chem. Soc.* 93:5542-5551 (1971); McLaughlin et al., *J. Biol. Chem.* 247:4382-4388 (1972); Lowe et al., *J. Biol. Chem.* 225:3944-3951 (1980); Roberts et al, *J. Biol. Chem.* 260:13502-13508 (1985); Roberts et al., *Arch. Biochem. Biophys.* 220:563-571 (1983), and Griffiths et al, *J. Biol. Chem.* 251:2049-2054 (1976)). The contents of all of the forementioned references are expressly incorporated by reference. Further to the forementioned references, Kaddurah-Daouk et al. (W092/08456; WO90/09192; U.S. 5,324,731; U.S. 5,321,030) also provide citations for the synthesis of a plurality of creatine analogs. The contents of all the aforementioned references and patents are incorporated herein by reference.

Creatine compounds which currently are available or have been synthesized include, for example, creatine, b-guanidinopropionic acid, guanidinoacetic acid, creatine phosphate disodium salt, cyclocreatine, homocyclocreatine, phosphinic creatine, homocreatine, ethylcreatine, cyclocreatine phosphate dilithium salt and guanidinoacetic acid phosphate disodium salt, among others.

Creatine phosphate compounds also can be synthesized chemically or enzymatically. The chemical synthesis is well known. Annesley, T.M. Walker, J.B., *Biochem. Biophys. Res. Commun.*, 74, 185-190(1977); Cramer, F., Scheiffele, E., Vollmar, A., *Chem. Ber.*, (1962), 95, 1670-1682.

Salts of the products may be exchanged to other salts using standard protocols. The enzymatic synthesis utilizes the creatine kinase enzyme, which is commercially available, to phosphorylate the creatine compounds. ATP is required by creatine kinase for phosphorylation, hence it needs to be continuously replenished to drive the reaction forward. It is necessary to couple the creatine kinase reaction to another reaction that generates ATP to drive it forward. The purity of the resulting compounds can be confirmed using known analytical techniques including ^{1}H NMR, ^{13}CNMR Spectra, Thin layer chromatography, HPLC and elemental analysis.

Existing Therapeutic Agents for Neurodegenerative Diseases

Therapeutic agents for treatment of neurodegenerative disease which are useful in combination with creatine compounds or creatine compounds and neuroprotective agents are described below.

Suitable therapeutic drugs for neurodegenerative diseases include those which have been approved by, for example, the United States Food and Drug Administration. Representative drugs useful in treatment of Alzheimer's disease include Cognex (tacrine) manufactured by Parke Davis which is a first generation acetylcholinesterase inhibitor and Aricept (donepizil) manufactured by Eisai which is a second generation acetylcholinesterase inhibitor.

Suitable drugs for treatment of Parkinson's Disease include Sinemet (carbidopa/levidopa) and Sinemet CR (carbidopa/levidopa sustained release) manufactured by DuPont Pharma. Levodopa is a metabolic precursor of dopamine that crosses the blood-brain barrier. Carbidopa inhibits conversion of levodopa before it crosses the blood-brain barrier. Permax (pergolide mesylate), manufactured by Athena, and Parlodel (bromocriptine mesylate), manufactured by Novartis, are therapeutic agents for treatment of Parkinson's Disease and are dopamine receptor agonists, often used as an adjunct to Sinemet. Eldepryl (selegiline), manufactured by Somerset, is yet another therapeutic agent for treatment of Parkinson's Disease and inhibits monoamine oxidase and is used as an adjunctive therapy. Symmetrel

(amantadine), manufactured by DuPont Pharma, has an unknown mechanism of treatment for Parkinson's Disease. Artane (trihexyphenidyl hydrochloride), manufactured by Lederle, also a suitable therapeutic agent is a muscarinic antagonist and is used as an adjunctive therapy.

An example of a therapeutic drug for treatment of ALS is Rilutek (riluzole), manufactured by Rhone-Poulenc Rorer. Rilutek elicits an inhibitory effect on glutanate release and has various neuroprotective effects, however, the mode of its action is unknown.

<u>Neuroprotective Agents Useful For Treating Nervous System Diseases</u>

Neuroprotective agents include those compositions which provide neuroprotection, e.g., approved drugs for the treatment or prevention of neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet, Sinmet CR, Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate excitotoxicity inhibitors (such as glutamate uptake and biosynthesis modulation with compounds like gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors; Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants, energy enhancers, vitamins and cofactors (such as spin traps, CoQ_{10}, carnitine, nicotinamide, Vitamin E or D) lipoic acid, vinpocetine.

<u>ATP Enhancing Agents Useful for Electron Transport</u>

ATP enhancing agents include those compounds which facilitate ATP production. These agents can be critical in the function of electron transport and oxidative phosphorylation and hence ATP production and neuronal cell survival. Examples include:

<u>Nicotinamide/Riboflavin:</u>

Riboflavin and nicotinamide are water soluble vitamins and components of coenzymes critical in the function of electron transport and oxidative phosphorylation and hence ATP production. The water soluble vitamins are referred to as the vitamin B complex. Riboflavin (vitamin B2) is a precursor of FAD, and niacin is the precursor of Nicotinamide adenine dinucleotide. Nicotinamide adenine dinucleotide is a major electron acceptor in the oxidation of fuel molecules. The reactive part of NAD+ is the

nicotinamide ring. In the oxidation of substrates the nicotinamide ring of NAD+ accepts a hydrogen ion and two electrons which are equivalent to a hydride ion. The reduced form of this carrier is called NADH. The other major electron carrier in the oxidation of fuel molecules is flavin adenine dinucleotide. FAD like NAD+ is a two electron acceptor. Hence the molecules riboflavin and nicotinamide are used as supplements to drive effectively oxidative phosphorylation and could have significant protective effects in stress conditions or disease states where energy production and oxidative phosphorylation are compromised.

Nicotinamide is a B vitamin and is a major component of NAD, and NADP which are critical components in the regulation of electron transport chain and energy production in the mitochondria. Nicotinamide is the amide of nicotinic acid, is a crystalline compound of the vitamin B complex, is convertible into nicotine acid in the body. Nicotinic acid is a group of vitamins of the B complex, central for growth and health in many animals and important in protein and carbohydrate metabolism. It is found in meat, liver, wheat germ, milk eggs. Also, Niacin is converted to nicotinamide in the body.

Treatment with nicotinamide in combination with riboflavin (Penn et.al., Neurology, 42: 2147-2152, 1992; Bernsen et.al., J. Neurol Sci. 118: 181-187, 1993) result in both biochemical and clinical improvement for patients with mitochondrial disorders. The combination of nicotinamide and coenzyme Q10 were shown to attenuate malonate induced energy defects and attenuate the striatial lesions produced by this compound, i.e., an animal model of Huntigton's disease (Beal et.al., Annals of Neurology, 26: 882-888, 1994). Amounts used were Q10 100-300 mg/kg/day, nicotinamide 500 mg/kg/day, and riboflavin 15 mg/kg/day.

Co-Enzyme Qs (CoQs):

A CoQs is a member of the family of co-enzyme Qs wherein the "s" is the number of isoprenoid units attached to the quinone ring. CoQ_{10} is a preferred CoQs of the present invention. CoQ_{10} is present in virtually all living cells. Although a molecular structure varies among different types of organisms, the chemical structure of CoQ_{10} (2,3 dimethoxy-5 methyl-6-decaprenyl benzoquinone) consists of a quinone ring (a molecular structure of carbon, hydrogen, and oxygen) with a long side chain. The body of the molecule is always the same but the number of the isoprene units (a 5 carbon chemical unit) attached to the quinone ring varies (human CoQ_{10} has 10 iso-prenoid units) the side chain is highly fat soluble which allows coq10 to lodge firmly in membranes inside

cells. CoQ_{10} is a large lipophilic fat soluble nutrient with a mol wt. of 862D. It is very soluble in chloroform and carbon tetrachloride and insoluble in water. CoQ_{10} is poorly absorbed unless it is specially prepared by solubilizing-emmulsifing in suitable oils or emmulsified in a silica base excipient containing a non-ionic surfactant. Multi approaches have been developed to enhance the bio-availability of the compound such as the use of oily preparations to bypass the liver.

CoQ_{10} is an essential nutrient that is a co-factor in the mitochondrial electron transport chain, the biochemical pathway in cellular respiration in which ATP and metabolic energy is derived, since all cellular functions depend on energy CoQ_{10} seems to be essential for the health of human tissue. Additionally, CoQ_{10} similar to Vitamin E, and K has anti-oxidant activity and scavenges free radical which could add to it's benefit to minimize injury for example to neuronal cells. Diets could be deficient in providing sufficient amounts of CoQ_{10} suggesting that supplementation with this compound could be of benefit in preserving tissue.

CoQ_{10} was first isolated from beef heart mitochondria by Dr. Frederick Crane in 1957 (Crane et al., Biochimica et Biophys. Acta, Vol25:220-221, 1957). In 1958 Prof. Karl Folkers and co-workers at Merck, Inc. determined the precise chemical structure of CoQ_{10}: 2,3 dimethoxy-5 methyl-6-decaprenyl benzoquinone, synthesized it and were the first to produce it by fermentation. In the mid 1960's Prof. Yamamura of Japan was the first to use CoQ_7 a related compound to treat a human disease (congestive heart failure). Multi clinical trials with CoQ_{10} followed.

Improved cardiovascular morbidity and mortality have been observed in several clinical studies using CoQ_{10} as a supplement (Serebruany et al., J. Cardiovascular Pharmacology 28(2):1775-181, 1996). Pretreatment with CoQ_{10} at 150 mg/day for 7 days suggested some protective benefit for patients undergoing routine vascular procedures requiring abdominal aortic cross clamping by attenuating the degree of peroxidative damage (Chello et al., J. of Cardiovascular Surgery 37(3):229-235, 1996). Benefit to patients with cardiomyopathy has been suggested with the use of CoQ_{10} at 100 mg/day for several weeks to years (Manzoli et al, It. J. Tiss. Reac. 12(3):173-178, 1990; Langsjoen. et al., Int. J. Tiss. Reac. 12(3):163-168, 1990; Langsjoen. et al., Am. J. Cardiol. (65):521-523, 1990, Langsjoen. et al., nt. J. Tiss. Reac. 12(3):169-171, 1990; Morisco et al., Clin Invest. 71:S134-S136, 1993).

Patients with mitochondrial myopathies placed on CoQ_{10} supplementation at 100-150 mg/day, for extended periods of time, showed benefit in reversing abnormal biochemical profiles and muscle function (Nakamura et al., Electromyography and Clinical Neurophysiology 35(6):365-370, 1995, Gold et al., Eur. Neurology 36(4):191-196, 1996, Ikerjiri et al. Neurol. 47(2):583-585, 1996). Also patients with mitochondrial myopathies secondary to HIV infection and treatment with AZT might benefit from CoQ_{10} supplementation (Dalakas et al., N Eng J Med. 322:1098-1105, 1990). Improved physical performance in patients with muscle dystrophies was noted upon supplementation with CoQ_{10} (Folkers et al., Biochimica et Biophysica Acta-Molecular Basis of Disease 1271(1):281-286, 1995). The combination of CoQ_{10} and Nicotinamide blocked striatal lesions produced by the mitochondrial toxin Malonate, an animal model of Huntington's Disease (Beal et al., Ann. Neuro 36(6):882-888, 1994). The combination of CoQ_{10} and Nicotinamide and free radical spin traps protected against MPTP neurotoxicity, an animal model of Parkinson's Disease (Schulz et al., Exp. Neurol. 132:279-283, 1995).

Free Radical Spin Traps:

Free radicals are formed as food and oxygen are metabolized to produce energy. These radicals can oxidize and kill cells. Oxidation is a chemical reaction in which a molecule transfers one or more electrons to another. Stable molecules usually have matched pairs of protons and electrons. In certain reactions, a free radical can be formed having unpaired electrons. Free radicals tend to be highly reactive, oxidizing agents. Free radicals can kill cells by damaging cell membranes, cytoskeleton and sensitive nuclear and mitochondrial DNA. Such intracellular damage can lead to the increase in calcium, increase in damaging proteases and nucleases and production of interferons, TNF-a and other tissue damaging mediators which lead to disease if overexpressed in response to oxidative stress. When free radicals interact with non-radicals, the result is usually a chain reaction. Only when two radicals meet or when antioxidants quench the reaction is the cascade of damage terminated. The most common reactive oxygen species (ROS) produced in vivo are hydrogen peroxide H_2O_2, hydroxyl OH, superoxide O_2, perhydroxyl HO_2, nitrogen oxide NO, and alkoxyl RO, and peroxyl ROO radicals.

In normal healthy individuals this process is offset by endogenous antioxidants and cellular repair mechanisms. However as organisms age and in certain diseases, the process can fall out of balance resulting in delitating and potentially fatal consequences. Oxidation is important factor in many diseases and disorders such as Parkinson's disease and Alzheimer's disease, ischemia reperfusion injury associated with stroke and heart

attack, and inflammatory conditions such as arthritis and ocular inflammation, AIDS dementia complex, inflammatory bowel disease and rational neovascularization, and multiple sclerosis.

Oxygen breathing animals have developed powerful antioxidant defense systems and cellular repair mechanisms to control this damage. Enzymes such as superoxide dismutase, catalse and glutathione peroxidase and vitamins such as tocopherol, ascorbate and carotene act to quench radical chain reactions. In general many of these natural molecules alone do not have great activity when given as supplements because they have to be produced within the cells to be effective in disease prevention.

Spin traps are chemical compounds that can protect cells from damaging effects of free radicals and hence slow or reverse the oxidation damage associated with these conditions. Suitable spin traps include PBN, S-PBN, DMPO, TEMPOL, azulenyl based spin traps, MDL, etc.

In an animal model of Parkinson's disease, nicotinamide or the free radical spin trap N-tert-a-(2-sulfophenyl) nitrone were effective in inhibiting moderate dopamine depletion (Schulz et al., Experimental Neurology 132, 279-283, 1995). In the same study, Q10 and nicotinamide protected against both mild and moderate depletion of dopamine. These results show that agents which improve mitochondrial energy production like Q10 and nicotinamide and the free radical scavengers can attenuate mild to moderate MPTP neurotoxicity.

Several free radical spin trap compounds can exert neuroprotective effects against both excitotoxicity and mitochondrial toxins *in vivo*.

L-Carnitine:

Carnitine is an important cofactor for normal cellular metabolism. Optimal utilization of fuel substrates for ATP generation is dependent on adequate carnitine stores. Fatty acids are activated on the outer mitochondrial membrane, whereas they are oxidized in the mitochondrial matrix. Long chain acyl CoA molecules do not readily traverse the inner mitochondrial membrane, and so a special transport mechanism is needed. Activated long chain fatty acids are carried across the inner mitochondrial membrane by carnitine. The acyl group is transferred from the sulfur atom of CoA to the hydroxyl group of carnitine to form acyl carnitine, which diffuses across the inner mitochondrial membrane. On the matrex side of this membrane the acyl group is

transferred back to CoA; which is thermodynamically feasible because of the O-acyl link in carnitine has high transfer potential. Oxidation of long chain fatty acids provides an excellent source of energy. Deficiencies of carnitine might result in impaired flow of metabolities form one compartment of a cell to another which can result in disease.

The supplementation of L-carnitine was shown to have some benefit to chronic hemodialysis patients. patients with cardiovascular diseases, muscle diseases, chronic fatigue, diabetic neuropathies, AIDS patients. Typical doses are 20-30 mg/Kg.

<u>Anti-oxidants:</u>

Anti-oxidants include those species of compounds which inhibit or prevent oxidation of tissues, such as vitamin E, alpha-omega fatty acids, BHP, ECGC, etc. such as those known in the art. Other anti-oxidants known in the art include pyruvate and lutein. Anti-oxidants can also be derived from natural sources such as berry meals and oils, e.g., from bilberries, elderberries, blackberries, blueberries, english hawthorn berries, red and black raspberries.

Reactive oxygen species are thought to be involved in a number of types of acute and chronic pathologic conditions in the brain and neural tissue. The metabolic antioxidant alpha-lipoate (thioctic acid, 1, 2-dithiolane-3-pentanoic acid; 1, 2-dithiolane-3 valeric acid; and 6, 8-dithiooctanoic acid) is a low molecular weight substance that is absorbed from the diet and crosses the blood-brain barrier. Alpha-lipoate is taken up and reduced in cells and tissues to dihydrolipoate, which is also exported to the extracellular medium; hence, protection is afforded to both intracellular and extracellular environments. Both alpha-lipoate and especially dihydrolipoate have been shown to be potent antioxidants, to regenerate through redox cycling other antioxidants like vitamin C and vitamin E, and to raise intracellular glutahione levels. Thus, it appears an ideal substance in the treatment of oxidative brain and neural disorders involving free-radical processes. Examination of current research reveals protective effects of these compounds in cerebral ischemia-reperfusion, excitotoxic amino acid brain injury, mitochondrial dysfunction, diabetes and diabetic neuropathy, inborn errors of metabolism, and other causes of acute or chronic damage to brain or neural tissue. Very few neuropharmacological intervention strategies are currently available for the treatment of stroke and numerous other brain disorders involving free radical injury. It is believed that the various metabolic antioxidant properties of alpha-lipoate relate to its possible therapeutic roles in a variety of brain and neuronal tissue pathologies: thiols are central to antioxidant defense in brain and other tissues. The most important thiol

antioxidant, glutahione, cannot be directly administered, whereas alpha-lipoic acid can. In vitro, animal, and preliminary human studies indicate that alpha-lipoate may be effective in numerous neurodegenerative disorders.

The term "herbal extracts" includes any fraction of an herb or other plant which can be administered to a subject. Preferrably, the herbal extract has neuroprotective activity. The term includes any part of the plant (e.g., leaves, seeds, stem, fruit, roots, etc.) which can be administered to a subject. Examples of herbal extracts include rosemary extract and black caraway seeds. Other examples compounds which may be included are extracts from green tea, licorice, tricosanthes, pau d'arco, gotu kola, barley grass, moss, kelp, garlic, astralagus, aloe vera, gingseng, ginko, cayenne, red clover flowers, apple, cherry, apricot, prune, hops, skullcap, valarian root, pomegranate, ashwagandha, borage, Bacopa Monniera, kava, grapes, citrus fruits (e.g., bioflavenoids), carob, ginger, wild milky oat, peppermint, blue-green algae, prickly ash, fo-ti, nutmeg, cardamon, reishi mushrooms, dong quai, kudzu, knotweed, yerba mate, lemon balm, tumeric, basil, vanilla, honey suckle, poria, perwinkle, codonopis, red peony, lycii berry, chrysanthanum, schizandra, moutan peony, adenophora, os draconis, wheat germ, tang kuai, tremella, eucommia, genetian, japanese plum, cherokee rose, olive oil, coffe bean, and chamomile.

Other neuroprotective agents which may advantageously be added to the compositions include phosphatidyl serine, acetyl-L-carnitine, huperzine A, melatonin, folic acid, choline, thiamin, riboflavin, niacin, biotin, calcium, iron, magnesium, potassium, zinc, iodine, inositol, dibencoside, copper, taurine, pentothenic acid, and phosphitidyl choline.

Utility

In the present invention, the combinations of creatine compounds and neuroprotective agents can be administered to an individual (e.g., a mammal), alone or in combination with another compound, for the treatment of diseases of the nervous system. As agents for the treatment of diseases of the nervous system, creatine compounds can interfere with creatine kinase/phosphocreatine functions, thereby preventing, ameliorating, arresting or eliminating direct and/or indirect effects of disease which contribute to symptoms such as paraplegia or memory impairment. Other compounds which can be administered together with the creatine compounds include neurotransmitters, neurotransmitter agonists or antagonists, steroids, corticosteroids

(such as prednisone or methyl prednisone) immunomodulating agents (such as beta-interferon), immunosuppressive agents (such as cyclophosphamide or azathioprine), nucleotide analogs, endogenous opioids, or other currently clinically used drugs. When co-administered with creatine compounds, these agents can augment interference with creatine kinase/phosphocreatine cellular functions, thereby preventing, reducing, or eliminating direct and/or indirect effects of disease.

A variety of diseases of the nervous system can be treated with creatine or creatine analogs in combination with neuroprotective agents, including but not limited to those diseases of the nervous system described in detail above. Others include bacterial or fungal infections of the nervous system. These creatine or analog combinations can be used to reduce the severity of a disease, reduce symptoms of primary disease episodes, or prevent or reduce the severity of recurrent active episodes. Creatine, creatine phosphate or analogs such as cyclocreatine and cyclocreatine phosphate can be used to treat progressive diseases. Many creatine analogs can cross the blood-brain barrier. For example, treatment can result in the reduction of tremors in Parkinson's disease, and other clinical symptoms.

Modes of Administration

The creatine compound and neuroprotective agent can be administered to the afflicted individual alone or in combination with another creatine analog or other agent. The combinations can be administered as pharmaceutically acceptable salts in a pharmaceutically acceptable carrier, for example. The combinations may be administered to the subject by a variety of routes, including, but not necessarily limited to, oral (dietary), transdermal, or parenteral (e.g., subcutaneous, intramuscular, intravenous injection, bolus or continuous infusion) routes of administration, for example. An effective amount (i.e., one that is sufficient to produce the desired effect in an individual) of a composition comprising a creatine analog and a neuroprotective agent is administered to the individual. The actual amount of drug to be administered will depend on factors such as the size and age of the individual, in addition to the severity of symptoms, other medical conditions and the desired aim of treatment.

Previous studies have described the administration and efficacy of creatine compounds *in vivo*. For example, creatine phosphate has been administered to patients with cardiac diseases by intravenous injection. Up to 8 grams/day were administered with no adverse side effects. The efficacy of selected creatine kinase

substrate analogs to sustain ATP levels or delay rigor during ischemic episodes in muscle has been investigated. On one study, cyclocreatine was fed to mice, rats and chicks, and appeared to be well-tolerated in these animals. Newly hatched chicks were fed a diet containing 1% cyclocreatine. In the presence of antibiotics, the chicks tolerated 1 % cyclocreatine without significant mortality, although the chicks grew more slowly than control chicks (Griffiths, G. R. and J. B. Walker, *J. Biol. Chem.* 251(7): 2049-2054 (1976)). In another study, mice were fed a diet containing 1% cyclocreatine for 10 days (Annesley, T. M. and J. B. Walker, *J. Biol. Chem.* 253(22): 8120-8125 (1978)). Cyclocreatine has been feed to mice at up to 1% of their diet for 2 weeks or for over 4 weeks without gross adverse effects. Lillie et al., *Cancer Res.*, 53: 3172-3178 (1993). Feeding animals cyclocreatine (e.g., 1% dietary) has been shown to lead to accumulation of cyclocreatine in different organs in mM concentrations. For example, cyclocreatine was reported to be taken up by muscle, heart and brain in rats receiving dietary 1% cyclocreatine. Griffiths, G. R. and J. B. Walker, *J. Biol. Chem.* 251(7): 2049-2054 (1976). As shown previously, antiviral activity of cyclocreatine is observed on administering 1% dietary cyclocreatine. Many of the above-referenced studies show that creatine analogs are been shown to be capable of crossing the blood-brain barrier.

The creatine compound and neuroprotective agent combination can be formulated according to the selected route of administration (e.g., powder, tablet, capsule, transdermal patch, implantable capsule, solution, emulsion). An appropriate composition comprising a creatine analog and neuroprotective agent can be prepared in a physiologically acceptable vehicle or carrier. For example, a composition in tablet form can include one or more additives such as a filler (e.g., lactose), a binder (e.g., gelatin, carboxymethylcellulose, gum arabic), a flavoring agent, a coloring agent, or coating material as desired. For solutions or emulsions in general, carriers may include aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride, solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. In addition, intravenous vehicles can include fluid and nutrient replenishers, and electrolyte replenishers, such as those based on Ringer's dextrose. Preservatives and other additives can also be present. For example, antimicrobial, antioxidant, chelating agents, and inert gases can be added. (See, generally, Remington's Pharmaceutical Sciences, 16th Edition, Mack, Ed., 1980).

The term "administration" is intended to include routes of administration which allow the creatine compound/neuroprotective agent to perform their intended function(s) of preventing, ameliorating, arresting, and/or eliminating disease(s) of the nervous system in a subject. Examples of routes of administration which may be used include injection (subcutaneous, intravenous, parenterally, intraperitoneally, etc.), oral, inhalation, transdermal, and rectal. Depending on the route of administration, the creatine/neuroprotective agent may be coated with or in a material to protect it from the natural conditions which may detrimentally effect its ability to perform its intended function. The administration of the creatine/neuroprotective agent is done at dosages and for periods of time effective to reduce, ameliorate or eliminate the symptoms of the nervous system disorder. Dosage regimes may be adjusted for purposes of improving the therapeutic or prophylactic response of the compound. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

In addition, the methods of the instant invention comprise creatine compounds effective in crossing the blood-brain barrier.

The creatine compounds/neuroprotective agents of this invention may be administered alone or as a mixture with other creatine compounds, or together with an adjuvant or other drug. For example, the creatine compound/neuroprotective agent may be coadministered with other different art-recognized moieties such as nucleotides, neurotransmitters, agonists or antagonists, steroids, immunomodulators, immunosuppressants, vitamins, endorphins or other drugs which act upon the nervous system or brain.

Creatine Kinase Isoenzymes in the Brain

Cells require energy to survive and to carry out the multitude of tasks that characterize biological activity. Cellular energy demand and supply are generally balanced and tightly regulated for economy and efficiency of energy use. Creatine kinase plays a key role in the energy metabolism of cells with intermittently high and fluctuating energy requirements such as skeletal and cardiac muscle, brain and neural tissues, including, for example, the retina, spermatozoa and electrocytes. As stated above, the enzyme catalyzes the reversible transfer of the phosphoryl group from creatine phosphate to ADP, to generate ATP. There are multi-isoforms of creatine

kinase (CK) which include muscle (CK-MM), brain (CK-BB) and mitochondrial (CK-Mia, CK-Mib) isoforms.

Experimental data suggest that CK is located near the sites in cells where energy generation occurs; e.g., where force generation by motor proteins takes place, next to ion pumps and transporters in membranes and where other ATP-dependent processes take place. It seems to play a complex multi-faceted role in cellular energy homeostasis. The creatine kinase system is involved in energy buffering/energy transport activities. It also is involved in regulating ADP and ATP levels intracellularly as well as ADP/ATP ratios. Proton buffering and production of inorganic phosphate are important parts of the system.

In the brain, this creatine kinase system is quite active. Regional variations in CK activity with comparably high levels in cerebellum were reported in studies using native isoenzyme electrophoresis, or enzymatic CK activity measurements in either tissue extracts or cultured brain cells. Chandler et al. *Stroke,* 19: 251-255 (1988), Maker et al. *Exp. Neurol.*, 38: 295-300 (1973), Manos et al. *J. Neurol. Chem.*, 56: 2101-2107 (1991). In particular, the molecular layer of the cerebellar cortex contains high levels of CK activity (Kahn *Histochem.*, 48: 29-32 (1976) consistent with the recent 3'P-NMR findings which indicate that gray matter shows a higher flux through the CK reaction and higher creatine phosphate concentrations as compared to white matter (Cadoux-Hudson et al. *FASEBJ.*, 3:2660-2666 (1989), but also high levels of CK activity were shown in cultured oligodendrocytes (Molloy et al. *J. Neurochem.*, 59:1925-1932 (1992), typical glial cells of the white matter. The brain CK isoenzyme CK-BB is the major isoform found in the brain. Lower amounts of muscle creatine kinase (CK-MM) and mitochondrial creatine kinase (CK-Mi) are found.

<u>Localization and Function of CK Isoenzymes in Different Cells of the Nervous System</u>

Brain CK (CK-BB) is found in all layers of the cerebellar cortex as well as in deeper nuclei of the cerebellum. It is most abundant in Bergmann glial cells (BGC) and astroglial cells, but is also found in basket cells and neurons in the deeper nuclei. Hemmer et al., *Eur. J. Neuroscience*, 6:538-549 (1994), Hemmer et al. *Dev. Neuroscience*, 15:3-5 (1993). The BGC is a specialized type of astroglial cell. It provides the migratory pathway for granule cell migration from the external to the

internal granule cell layer during cerebellar development. Another main function of these cells is the proposed ATP dependent spatial buffering of potassium ions released during the electrical activity of neurons (Newman et al. *Trends Neuroscience*, 8:156-159 (1985), Reichenbach, *Acad. Sci New York*, (1991), 272-286. Hence, CK-BB seems to be providing energy (ATP) for migration as well as K^+ buffering through regulation of the Na^+/K^+ ATPase. The presence of CK-BB in astrocytes may be related to the energy requirements of these cells for metabolic interactions with neurons; e.g., tricarboxylic acid cycle (TCA) metabolite and neurotransmitter trafficking. Hertz, *Can J. Physiol. Pharmacol.*, 70: 5145-5157 (1991).

The Purkinje neurons of the cerebellum play a very important role in brain function. They receive excitatory input from parallel fibers and climbing fibers, they represent the sole neuronal output structures of the cerebellar cortex. Calcium mediated depolarizations in Purkinje cell dendrites are thought to play a central role in the mechanism of cerebellar motoric learning. Ito *Corr. Opin. Neurobiol.*, 1:616-620 (1991). High levels of muscle CK (CK-MM) were found in Purkinje neurons. There is strong evidence to support that CK-MM is directly or indirectly coupled to energetic processes needed for Ca^{++} homeostasis or to cellular processes triggered by this second messenger.

The glomerular structures of the cerebellum contain high levels of CK-BB and mitochondrial CK (CK-Mi). Large amounts of energy are needed in these structures for restoration of potassium ion gradients partially broken down during neuronal excitation as well as for metabolic and neurotransmitter trafficking between glial cells and neurons. The presence of CK in these structures may be an indication that part of the energy consumed in these giant complexes might be supported by the creatine kinase system.

In neurons, CK-BB is found in association with synaptic vesicles (Friedhoff and Lerner, *Life Sci.*, 20:867-872 (1977) as well as with plasma membranes (Lim et al., *J. Neurochem.*, 41: 1177-1182 (1983)).

There is evidence to suggest that CK is bound to synaptic vesicles and to the plasma membrane in neurons may be involved in neurotransmitter release as well as in the maintenance of membrane potentials and the restoration of ion gradients before and after stimulation. This is consistent with the fact that high energy turnover and

concomitantly high CK concentrations have been found in those regions of the brain that are rich in synaptic connections; e.g., in the molecular layer of the cerebellum, in the glomerular structures of the granule layer and also in the hippocampus. The observation that a rise in CK levels observed in a fraction of brain containing nerve endings and synapses, parallels the neonatal increase in Na^+/K^+ ATPase is also suggestive that higher levels of creatine phosphates and CK are characteristic of regions in which energy expenditure for processes such as ion pumping are large. Erecinska and Silver, *J. Cerebr. Blood Flow and Metabolism*, 9:2-l9 (1989). In addition, protein phosphorylation which plays an important role in brain function is also through to consume a sizable fraction of the total energy available in those cells (Erecinska and Silver, *id.* 1989). Finally, CK, together with nerve-specific enolase belongs to a group of proteins known as slow component b (SCb). These proteins are synthesized in neuronal cell body and are directed by axonal transport to the axonal extremities. Brady and Lasek, *Cell*, 23: 515-523 (1981), Oblinger et al., *J. Neurol.*, 7: 433-462 (1987) The question of whether CK participates in the actual energetics of axonal transport remains to be answered.

In conclusion, the CK system plays a key role in the energetics of the adult brain. This is supported by ^{31}P NMR magnetization transfer measurements showing that the pseudo first order rate constant of the CK reaction in the direction of ATP synthesis as well as CK flux correlate with brain activity which is measured by EEG as well as by the amount of deoxyglucose phosphate formed in the brain after administration of deoxyglucose. The present inventors have discovered that diseases of the nervous system can be treated by modulating the activity of the creatine kinase/creatine phosphate pathway.

The Role of Creatine Kinase in Treating Diseases of the Nervous System

The mechanisms by which nerve cell metabolites are normally directed to specific cell tasks is poorly understood. It is thought that nerve cells, like other cells, regulate the rate of energy production in response to demand. The creatine kinase system is active in many cells of the nervous system and is thought to play a role in the allocation of high energy phosphate to many diverse neurological processes, such as neurotransmitter biosynthesis, electrolyte flux and synaptic communication. Neurological function requires significant energy and creatine kinase appears to play an important role in controlling the flow of energy inside specialized excitable cells such as neurons. The induction of creatine kinase, the BB isozyme and the brain mitochondrial creatine kinase in particular, results in the generation of a high energy

state which could sustain or multiply the pathological process in diseases of the nervous system. Creatine kinase induction also causes release of abnormally elevated cellular energy reserves which appear to be associated with certain diseases of the nervous system. Conversely, suppression of the creatine kinase system, or aberrances in it, induce a low energy state which could result in or assist in the death in the process of all the nervous system.

The components of the creatine kinase/phosphocreatine system include the enzyme creatine kinase, the substrates creatine and creatine phosphate, and the transporter of creatine. Some of the functions associated with this system include efficient regeneration of energy in cells with fluctuating and high energy demand, phosphoryl transfer activity, ion transport regulation, cytoskeletal association, nucleotide pool preservation, proton buffering, and involvement in signal transduction pathways. The creatine kinase/phosphocreatine system has been shown to be active in neurons, astrocytes, oligodendrocytes, and Schwann cells. The activity of the enzyme has been shown to be up-regulated during regeneration and down-regulated in degenerative states, and aberrant in mitochondrial diseases.

Many diseases of the nervous system are thought to be associated with abnormalities in an energy state which could result in imbalanced ion transport neurotransmitter release and result in cell death. It has been reported that defects in mitochondrial respiration enzymes and glycolytic enzymes may cause impairment of cell function.

Without wishing to be bound by theory, it is thought that if the induction or inhibition of creatine kinase is a cause or a consequence of disease, modulating its activity, may block the disease. Modulating its activity would modulate energy flow and affect cell function. Alternatively, another possibility is that creatine kinase activity generates a product which affects neurological function. For example, creatine phosphate may donate a phosphate to a protein to modify its function (e.g., activity, location). If phosphocreatine is such a phosphate donor, creatine analogs which are phosphorylatable or phosphocreatine analogs may competitively inhibit the interaction of phosphocreatine with a target protein thereby directly or indirectly interfering with nervous system functions. Alternatively, phosphorylatable creatine analogs with altered phosphoryl group transfer potential may tie up phosphate stores preventing efficient transfer of phosphate to targets. A neurological disease could be associated with down regulation of creatine kinase activity. In such cases,

replenishment of the substrates, e.g., creatine, creatine phosphate or a substrate analog, which could sustain ATP production for an extended of time, with other activators of the enzyme could be beneficial for treatment of the disease.

Ingestion of creatine analogs has been shown to result in replacement of tissue phosphocreatine pools by synthetic phosphagens with different kinetic and thermodynamic properties. This results in subtle changes of intracellular energy metabolism, including the increase of total reserves of high energy phosphate (see refs. Roberts, J.J. and J.B. Walker, *Arch Biochem. Biophys* 220(2): 563-571 (1983)). The replacement of phosphocreatine pools with slower acting synthetic phosphagens, such as creatine analogs might benefit neurological disorders by providing a longer lasting source of energy. One such analog, cyclocreatine (1-carboxymethyl-2-aminoimidazolidine) modifies the flow of energy of cells in stress and may interfere with ATP utilization at sites of cellular work.

The pathogenesis of nerve cell death in neurodegenerative diseases is unknown. A significant amount of data has supported the hypothesis that an impairment of energy metabolism may underlie the slow exitotoxic neuronal death. Several studies have demonstrated mitochondrial or oxidative defects in neurodegenerative diseases. Impaired energy metabolism results in decreases in high energy phosphate stores and a deteriorating membrane potential. Under these conditions the voltage sensitive Mg2+block of NMDA receptors is relieved, allowing the receptors to be persistently activated by endogenous concentrations of glutamate. In this way, energy related metabolic defects may lead to neuronal death by a slow exitotoxic mechanism. Recent studies indicate that such a mechanism occurs *in vivo,* and it may play a role in animal models of Huntington's disease and Parkinson's disease.

As discussed in detail above, the creatine kinase/ creatine phosphate energy system is only one component of an elaborate energy-generating system found in the nervous system. The reaction catalyzed by this system results in the rapid regeneration of energy in the form of ATP at sites of cellular work. In the mitochondria the enzyme is linked to the oxidative phosphorylation pathway that has been implicated in diseases of the nervous system. There the enzyme works in the reverse direction where it stores energy in the form of creatine phosphate.

The invention is further illustrated in the following examples which in no way should be construed as being further limiting. These examples provide evidence that creatine compounds, represented by creatine itself and the analogue cyclocreatine, are neuroprotective agents in animal models used for neurodegenerative diseases, specifically, Huntington's disease and Parkinson's disease. The contents of all references, pending patent applications and published patent applications, cited throughout this application (including the background section) are hereby incorporated by reference. For example, all teachings with regard to creatine compounds, ATP enhancing agents, neuroprotective agents, etc. are intended to be part of the present invention. It should be understood that the models used throughout the examples are accepted models and that the demonstration of efficacy in these models is predictive of efficacy in humans.

Examples

Example 1: Models for Huntington's Disease: Malonate and 3-Nitropropionic Acid

There is substantial evidence that energy production may play a role in the pathogenesis of neurodegenerative diseases (Beal et al., *Ann. Neurol.* 31:119-130 (1992)). Impaired energy production may lead to activation of excitatory amino acid receptors, increases in intracellular calcium and the generation of free radicals (Beal et al., *Ann. Neurol.* 38:357-366 (1995)). In Huntington's Disease (HD) there is reduced mitochondrial complex II-III activity in post mortem tissue and increased cerebral lactate concentrations *in vivo* (Browne et al., *Ann. Neurol.*, in press, (1997); Gu et al., *Ann. Neurol.* 39:385-389 (1996); Jenkins et al., *Neurology* 43 :2689-2695 (1993)).

Animal models of Huntington's disease involve defects in energy production. Malonate and 3-nitropropionic acid (3-NP) are, respectively, reversible and irreversible inhibitors of complex II (succinate dehydrogenase) which produce striatal lesions similar to those of HD (Beal et al., *J. Neurochem.* 61:1147-1150 (1993); Brouillet et al., *PNAS* 92:7105-7109 (1995); Henshaw et al., *Brain Research* 647:161-166 (1994)). The pathogenesis of lesions produced by these compounds involves energy depletion, followed by activation of excitatory amino acid receptors and free radical production (Schulz et al., *J. Neurosci.* 15:8419-8429 (1995); Schulz et al., *J. Neurochem.* 64:936-939 (1995)).

The enzyme succinate dehydrogenase plays a central role in both the tricarboxylic acid cycle and the electron transport chain in the mitochondria. Intrastriatal injections of malonate in rats were shown to produce dose dependent striatal excitotoxic lesions which are attenuated by both competitive and non-competitive NMDA antagonists (Henshaw et al., *Brain Res.* 647:161-166 (1994)). Furthermore, the glutamate release inhibitor lamotrigine also attenuates the lesions. Co-injection with succinate blocks the lesions consistent with an effect on succinate dehydrogenase. The lesions are accompanied by a significant reduction of ATP levels as well as significant increase in lactate levels *in vivo* as shown by chemical shift resonance imaging (Beal et al., *J. Neurochem* 61:1147-1150 (1993)). Furthermore, the increases in lactate are greater in older animals consistent with a marked age of the lesion. Histological studies have shown that the lesion spares NADPH-diaphorase neurons. Somatostatin concentrations were also spared. *In vivo* magnetic resonance imaging of lesions shows a significant correlation between increasing lesion size and lactate production.

A series of experiments demonstrated that the administration of Q 10 or nicotinimide produced dose dependent protection against the lesions in the malonate animal model. These compounds attenuated ATP depletion produced by malonate *in vivo.* Furthermore, the co-administration of Q 10 with nicotinimide attenuated the lesions and reduced increases in lactate which occurred after intrastriatal malonate injections.

All of the above mentioned studies supported malonate and 3-NP as useful models for the neuropathologic and neurochemical features of HD. The lesions produced similar patterns of cellular sparing seen in HD. There is a depletion of striatal spiny neurons, yet a relative preservation of the NADPH diaphorase interneurons. Furthermore, there is an increase in lactate concentration which has been observed in HD.

Oral administration of creatine and its analogue cyclocreatine were examined to determine their ability to attenuate malonate lesions. Creatine was administered orally to rats in their feed at doses of 0.25-3.0% of the diet. Cyclocreatine was administered at 0.2-1.0%. Controls received unsupplemented otherwise identical diets. The compounds were administered for two weeks prior to the administration of malonate and then for a further week prior to sacrifice. Malonate was dissolved in distilled deionized water and the pH wad adjusted to 7.4 with 0.1 m HCI.

Intrastriatal injections of 1.5 ul of malonate containing 3 μmol were made into the striatum at the level of the bregma 2.4 mm lateral to the midline and 4.5 mm ventral to the dura. Animals were sacrificed at 7 days by decapitation, and the brains were quickly removed and placed in ice cold 0.9% saline solution. Brains were sectioned at 2 mm intervals. Slices were then placed posterior side down in 2% 2,3,5-triphenyltetrazolium chloride. Slices were stained in the dark at room temperature for 30 minutes and then removed and placed in 4% paraformaldehyde, pH 7.3. Lesions, noted by pale staining, were evaluated on the posterior surface of each section using a Bioquant 4 system by an experienced histologist blinded to experimental conditions. These measurements have been validated by comparing them to measurements obtained on adjacent Nissl stain sections.

It was found that oral supplementation with both creatine and cyclocreatine protected against striatal malonate lesions. A dose response curve for neuroprotection by both creatine and cyclocreatine against malonate induced striatal lesions was then examined. As shown in Figure 2, increasing doses of creatine from 0.25-3% in the diet exerted dose dependent neuroprotective effects against malonate induced striatal lesions. Significant protection occurred with doses of 1% and 2% in the diet. There was less protection at 3% creatine, suggesting that a U shaped dose response may occur with higher doses. Administration of cyclocreatine resulted in dose dependent neuroprotective effects which were significant at a dose of 1% cyclocreatine.

In the 3-NP model, creatine was administered orally at a dose of 1% in feed. Controls received unsupplemented rat chow. 3-NP was diluted in water and adjusted to pH 7.4 with NaOH and administered at a dose of 10 mg/Kg intraperitoneally every 12 hours. Animals became acutely ill after 9-11 days. Since there was variability in the times at which animals became ill, they were clinically examined 3 hours after the injections and 1 animal of each group was sacrificed when an animal was acutely ill, regardless of whether it was on a control diet or a creatine supplemented diet (Schulz et al., J. Neurochem. 64:936-939 (1995)). Nine to ten animals were examined in each group. Animals were sacrificed after showing acute illness and striatal lesion volume was assessed by TTC staining as above. Statistical comparison was made by student's t test.

A remarkable level of neuroprotection was seen against subacute 3-NP neurotoxicity in creatine treated animals, as shown in Figure 3. Dietary

supplementation with 1% creatine resulted in significant 83% reduction in lesion volume produced by 3-NP. This suggests that dietary supplementation with creatine may exert its greatest efficacy against more slowly evolving metabolic insults than against acute insults.

Example 2: MPTP as a model for Parkinson's Disease

MPTP, or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine is a neurotoxin which produces a Parkinsonian syndrome in both man and experimental animals. The initial report was by a chemist who was synthesizing and self injecting an opiate analogue. He inadvertently synthesized MPTP and developed profound Parkinsonism. Subsequent pathologic studies showed severe degeneration in the pars compacta of the substantia nigra. A large outbreak subsequently occurred in California. These patients developed typical symptoms of Parkinsonism. They also had positron emission tomography done which showed a marked loss of dopaminergic innervation of the striatum.

Studies of the mechanism of MPTP neurotoxicity show that it involves the generation of a major metabolite, MPP^{+}. This metabolite is formed by the activity of monoamine oxidase on MPTP. Inhibitors of monoamine oxidase block the neurotoxicity of MPTP in both mice and primates. The specificity of the neurotoxic effects of MPP+ for dopaminergic neurons appears to be due to the uptake of MPP+ by the synaptic dopamine transporter. Blockers of this transporter prevent MPP+ neurotoxicity. MPP+ has been shown to be a relatively specific inhibitor of mitochondrial complex I activity. It binds to complex I at the retenone binding site. In *vitro* studies show that it produces an impairment of oxidative phosphorylation. *In vivo* studies have shown that MPTP can deplete striatal ATP concentrations in mice. It has been demonstrated that MPP+ administered intrastriatally in rats produces significant depletion of ATP as well as increases in lactate confined to the striatum at the site of the injections. The present inventors have recently demonstrated that coenzyme Q_{10}, which enhances ATP production, can significantly protect against MPTP toxicity in mice.

The effect of two representative creatine compounds, creatine and cyclocreatine, were evaluated using this model. Creatine and cyclocreatine were administered in the initial pilot experiment as 1% formulation in the feed of animals, and was administered for three weeks before MPTP treatment. MPTP was administered intraperitoneally at a dose of 15mg/kg every 2 hours for five injections.

The animals then remained on either creatine or cyclocreatine supplemented diets for 1 week before sacrifice. The mice examined were male Swiss Webster mice weighing 30-35 grams obtained from Taconic Farms. Control groups received either normal saline or MPTP hydrochloride alone. MPTP was administered in 0.1 ml of water. The MPTP was obtained from Research Biochemicals. Eight to twelve animals were examined in each group. Following sacrifice the two striata were rapidly dissected and placed in chilled 0.1 M perchloric acid. Tissue was subsequently sonicated, and aliquots were taken for protein quantification using a fluorometer assay. Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were quantified by HPLC with 16 electrode electrochemical detection. Concentrations of dopamine and metabolites were expressed as nmol/mg protein. The statistical significance of differences was determined by one-way ANOVA followed by Fisher PLSDpost-hoc test to compare group means.

The initial results are shown in Figure 4. Oral administration of either cyclocreatine or creatine significantly protected against DOPAC depletions induced by MPTP. Cyclocreatine was effective against MPTP induced depletions of homovanillic acid. Both administration of creatine and cyclocreatine produce significant neuroprotection against MPTP induced dopamine depletions. The neuroprotective effect produced by cyclocreatine was greater than that seen with creatine alone.

A dose response study was conducted where the creatine dose was 0.25%-3.0% of the diet and cyclocreatine 0.25-1.0% of the diet. The results, shown in Figure 5, demonstrate that doses of 0.25%, 0.5% and 1.0% creatine exerted dose-dependent significant neuroprotection effects which disappeared at doses of 2.0% and 3.0% creatine, consistent with a U shaped dose response curve. Cyclocreatine exerted significant protection against dopamine depletions at 0.5% and 1.0% cyclocreatine. Effects of creatine on the dopamine metabolites homovanillic acid (HVA) and 3-4-dihydroxyphenyl acetic acid (DOPAC) paralleled those seen with dopamine. Cyclocreatine also exerted neuroprotection effects against HVA and DOPAC, although protection against HVA depletion was not seen with 0.5% cyclocreatine which was suspected to be due to experimental variability.

These results indicate that the administration of creatine or cyclocreatine can produce significant neuroprotective effects against MPTP induced dopaminiergic

toxicity. These results imply that these compounds are useful for the treatment of Parkinson's disease. The data further establish the importance of the creatine kinase system in buffering energy and survival of neuronal tissue. Therefore, creatine compounds which can sustain energy production in neurons are going to emerge as a new class of protective agents of benefit therapeutically in the treatment of neurodegenerative diseases where impairment of energy has been established.

Example 3: Effect of Dietary Creatine in a Mouse Model for ALS

Motor neuron degeneration was generated in mice that express a human Cu, Zn superoxide dismutase mutation. Gurney et al., *Science,* vol. 264, pp 1772-1775 (1994) These FALS mice develop a syndrome which mimics the symptoms of familial amyotropic lateral sclerosis (FALS). Gradual loss of motor function becomes apparent, and typically the mice do not survive beyond 140 days.

FALS mice were divided into control and test groups. At approximately 80 days (between 70 and 90 days) after birth, the test groups (containing 5 mice per group) were changed over from a standard diet to a diet containing 1% creatine. The control group (containing 6 mice per group) were fed the standard diet.

Behavioral Testing-Rotorod

Mice were given two days to become aquatinted with the rotorod apparatus before testing began. Testing began with the animals trying to stay on a rod that was rotating at 1 rpm. The speed was then increased by 1 rpm every 10 seconds until the animal fell off. The speed of rod rotation at which the mouse fell off was used as the measure of competency on this task. Animals were tested every other day until they could no longer perform the task

The results for the test and control animals are shown in Figure 3. As shown in the Figure, the creatine-fed animals showed significantly better performance throughout the experiment suggesting less degeneration of motoneural skills than the control mice which were fed a standard diet.

Survival

FALS mice begin to show behavioral symptoms at about 120 days. The initial symptom is high frequency resting tremor. This progresses to gait abnormalities and uncoordinated movements. Later, the mice begin to show hemiparalysis of the hindlimbs, eventually progressing to paralysis of the forelimbs

and finally, complete paralysis. Animals in this study were sacrificed when they could no longer roll over within 10 seconds of being pushed on their side. This time point was taken as the time of death.

The results are shown graphically in Figure 4. Figure 4 shows that the animals placed on a diet containing 1% creatine survived longer than those placed on the control diet. Over 14 days of extension in survival was noted, which is a statistically significant improvement over the control mice.

The experiments performed on the FALS mice demonstrate that creatine has beneficial effects as an additional therapy for ALS. It improves the quality of life and extends survival.

Example 4: Neuroprotective Effects of Creatine and Nicotinamide against NMDA Mediated Excitotoxic Lesions

Materials and Methods

Studies of the neuroprotective effects of creatine and nicotinamide were carried out in 250 to 300 g male Sprague-Dawley rats. Creatine was administered orally to rats in their feed at a dose of 1% in the diet. Nicotinamide was administered orally with apple juice at a dose of 0.5% in the drinking water. Rats were treated for one week prior to intracerebral injectior~s. Animals then remained on the control or supplemented diets for one week prior to being sacrificed. Eleven to 12 animals were examined in each experimental group. NMDA was administered at a doge of 240 nmol in 1 µl. AMPA was administered at a dose of 30 nmol in 1 µl and kainic acid was administered at dose of 5 nmol in 1 µl. Malonate was dissolved in distilled deionized water and the pH was adjusted to 7.4 with HCI. Intrastriatal injections of 3 µmol of malonate in 1.5 µl were made with a 10 µl Hamiiton syringe fitted with a 26 gauge blunt tip needle, into the left striatum at the level of the bregma, 2.4 mm lateral to the midline and 4.5 mm ventral to the dura as described previously [Matthews, R.T *et al.* J. *Neurosci.*, 18 (1998) 156-163]. Following sacrifice the brains were quickly removed and placed in ice cold 0.9% saline solution. Brains were sectioned at 2 mm intervals throughout the rostro-caudal axis of the striatum. Slices were then placed posterior side down in 2% 2,3,5-triphenyltetrazolium chloride (TTC). Slices were stained in the dark at room temperature for 30 min and then removed and placed in 4% paraformaldehyde, pH 7.3. Lesions noted by pale staining were evaluated on the posterior surface of each section using a Bioquant 4 system, which calculates the volume of the lesions in each section,

by an experienced histologist blinded to experimental conditions. These measurements have been validated by comparing them with measurements obtained on adjacent Nissl stained sections. Statistical comparisons were made by unpaired t tests or by one-way analysis of variance followed by Fisher's protected least significant difference for post-hoc comparisons.

RESULTS

Creatine administration produced significant neuroprotective effects against striatal lesions produced by NMDA. There was no significant protection against either kainic acid or AMPA induced striatal excitotoxic lesions. Administration of nicotinamide alone produced a reduction in striatal lesion volume, however the reduction did not reach significance. Administration of creatine alone produced a significant neuroprotective effect against malonate lesions. The administration of nicotinamide with creatine produced additive neuroprotective effects which were greater than those seen with either creatine or nicotinamide alone.

Previous studies have demonstrated that NMDA excitotoxic lesions are associated with impairment of both ATP and phosphocreatine levels [Bordelon *et al.* J *Neurochem*, 69 (1997) 1629-1639, Mitani, A., *et al.* J *Neurochem,* 62 (1994) 626-634]. There is also data that kainc acid lesions are associated with energy impairment. Lesions produced by NMDA however appear to be linked to rnechanisms which differ from those which are associated with AMPA and kainic acid toxicity. An increase in calcium via activation of NMDA receptor is much more toxic than comparable increases caused by activation of voltage active calcium channels or kainic acid receptors (Tymianski *et al. J Neurosci, 13* (1993) 2085-2104). Furthermore increased intracellular calcium following activation of NMDA receptors is associated with a much greater increase in free radical production than comparable increases produced by activation of kainate receptors or voltage dependent calcium channels (Dugan *et al. J. Neurosci.,* 15 (1995) 6377-6388, Reynolds *et al.* J Neurosci, 15 (1995) 3318-3327). Activation of NMDA receptors is tied to a more rapid uptake of calcium into the mitochondria as compared to activation by voltage dependent calcium channels or by activation of AMPA or kainic acid receptors (Peng *et al. Mol Pharmacol,* 53 (1998) 974-980). Nitric oxide synthase inhibitors are effective in blocking NMDA excitotoxicity both in vitro and in vivo, whereas they are ineffective against both kainic acid and AMPA toxicity (Dawson *et al. Neurosci, 13 (1993)* 2651-2661). Specific coupling of NMDA receptors to nitric oxide neurotoxicity occurs by the NMDA receptor scaffolding protein PSD-95 (post-synaptic density-95) (Sattler *et al. Science,* 284 (1999) 1845-1848). Suppressing the expression of

PSD-95 attenuates excitotoxicity triggered by NMDA receptors, but not that produced by other glutamate receptors or calcium ion channels.

Creatine kinase along with its substrates creatine and phosphocreatine constitute an intricate cellular energy buffering and transport system connecting sites of energy production with sites of energy consumption (Hemmor *Dev. Neurosci.,* 15 (1993) 249-260). Creatine administration also stabilizes the mitochondrial creatine kinase and inhibits opening of the mitochondrial transition pore (O'Gorman *et al. FEBS* Lett., 414 (1997) 253-2571). Creatine administration can also stimulate mitochondrial respiration and phosphocreatine synthesis (O'Gorman *et al. Biochim Biophys Acta,* 1276 (1996) 161-170). Phosphocreatine diffuses to the cytoplasm where it serves as both a temporal and spatial energy buffer maintaining ATP levels utilized by the sodium potassium ATPase and the calcium ATPase. Its importance to brain function is supported by *in vivo*^{31}P NMR transfer measurements showing correlations of creatine kinase flux with brain activity as measured both by the EEG as well as brain 2deoxyglucose uptake (Corbett *et al J. Cereb. Blood Flow Metab.,* 14 (1994)1070-1077, Sauter *et al.* J. *Biol. Chem.,* 268 (1993) 13166-13171). Another potential mechanism by which phosphocreatine could inhibit excitotoxicity is by increasing glutamate uptake. Phosphocreatine serves as a direct energy source for glutamate uptake into synaptic vesicles (Xu *et al.J. Biol. Chem.,* 271 (1996) 13435-1344028). Lastly creatine kinase appears to be coupled directly or indirectly to energetic processes required for calcium homeostasis (Steeghs *et al. Cell,* 89 (1997) 93- 103). Creatine pretreatment delayed increases in intracellular calcium produced by 3-nitropropionic acid in cortical and striatal astrocytes *in vivo* (Deshpande *et al.Exp. Neurol.,* 145 (1997) 38-45). Administration of creatine may therefore improve intracellular calcium buffering and prevent free radical production by mitochondria. Creatine also protects mitochondrial creatine kinase from inactivation by peroxynitrite which is implicated in excitotoxic cell death (Stachowiak *et al. J Biol Chem,* 273 (1998)16694-16699). The present results suggest that stabilization of mitochondria and increasing mitochondrial PCr synthesis may be particularly effective against NMDA excitotoxicity as compared with that produced by nonNMDA receptor activation.

In the present study, it was also examined whether creatine could exert additive neuroprotective effects in combination with nicotinamide. It was found that creatine produced significant neuroprotective effects against malonate. A small protective effect of nicotinamide alone was found, although it did not reach statistical significance. The combination of nicotinamide with creatine however was more efficacious than the administration of either nicotinamide or creatine alone. Not to be limited by theory,

nicotinamide may be exerting neuroprotective effects either by increasing brain levels of NADH which is a cofactor of the electron transport chain, or by inhibiting the activation of polyADP-ribose polymerase which can lead to a depletion of intracellular ATP levels. Creatine is neuroprotective against 3-nitropropionic acid and MPTP toxicity, and that creatine significantly extends survival in a transgenic mouse model of ALS (Klivenyi *et al. Nature* Med., 5 (1999) 347-350, Matthews *et al. Exp Neurol,* 157 (1999) 142-149). The present studies provide further evidence that creatine exerts neuroprotective effects *in vivo*. Oral supplementation with creatine or creatine in combination with nicotinamide may therefore represent a novel therapeutic strategy for a number of neurodegenerative diseases.

Creatine administration may be able to increase intracellular energy stores and to inhibit activation of mitochondrial permeability transition. It was found that administration of creatine in the diet significantly protected against NMDA excitotoxic lesions. In addition, creatine produced significant protection against malonate induced striatal lesions and exerted additive effects against these lesions when combined with nicotinamide.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.